PROCEEDINGS

of the

6th INTERNATIONAL POULTRY MEAT CONGRESS

Organised by BESD-BİR

1-5 March 2023 Titanic Deluxe Golf Belek - ANTALYA - TURKEY

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6th INTERNATIONAL POULTRY MEAT CONGRESS

1-5 March 2023 Titanic Deluxe Golf Belek - ANTALYA - TURKEY

FULL TEXT BOOK

Association of Poultry Meat Producers and Breeders (BESD-BİR)

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DEAR DISTINGUISHED STAKEHOLDERS OF THE POULTRY MEAT INDUSTRY AND FRIENDS,

We are happy and excited to announce that the 6th International Poultry Meat Congress (UBEK-6), which has been postponed for about 2 years, due to the COVID-19 pandemic, will be held at 1-5 March 2023 in Antalya Titanic Deluxe Hotel. We are proud and happy to held our congress in 2023 in harmony with the 100th anniversary of The Republic of Turkey and the 30th anniversary of The Turkish Poultry Meat Producers and Breeders Association (BESD-BİR).

We, as BESD-BIR, would like to thank to you and to our honorable stakeholders for the interest and for the support that shown to our Poultry Meat Congress which we started to organize in 2011 and become an important brand on the international platform, together with you.

The 5th (UBEK-5) achieved an important success with the participation of approximately 1.500 attendees from 32 countries, including both poultry research scientists and practitioners, leading speakers from across the world. The leading scientists and experts shared with us the latest technological and scientific developments applied in the world and made very valuable contributions to the poultry meat industry.

Now, we will continue from where we left off and we started preparations with a strong motivation to raise the congress up to higher levels. Our ambition is to welcome you with highly advanced contents in the 6th UBEK in 2023. in Antalya which one of the most beautiful mediterranean cities.

All countries that have been exposed to the disruption of global trade, continued to population growth, and the negative effects of climate change on agriculture during the Covid 19 pandemic in the world, we have better understood the importance of agriculture and food safety. Just after this period, the war between Russia and Ukraine affects our life in a way that causes very important risks and problems for all humanity, and changed our perspective on the future.

In the new world order, sustainable production and efficiency, self-sufficiency, local production and logistics service have become the most valuable issues for the poultry meat sector as well as for all sectors. In particular, excessively rising commodity prices and supply problems are the most important risks that will negatively affect production, and there is a need to develop alternative and different strategies. In the light of these determinations. As poultry producers, with a proactive approach, it is essential that we must prepare for the new normal and the for what future will bring. At the 6th UBEK, as the first preparation for this period, we will plan our own roadmap by discussing the worldwide challenges, the solutions and the new visions.

Turkey's poultry meat production increased by 39% from 1.6 million tons to 2. 3 million tons in a short period of 10 years (2011-2021) and exports increased by 154 % from 248 thousand tons to 632 thousand tons. It is clear that the Turkish Poultry Meat Industry makes valuable contributions to healthy, adequate and stable food supply, not only for people living in Turkey but also for people living in other countries.

In the first week of the spring of 2023, we have the great pleasure to invite you for the 6th UBEK in Antalya, to take the steps that will benefit the sector together in order to contribute more to the humanity. Many thanks to all of those who will contribute and join us for making the congress possible.

We wish you healthy days and success with the most sincere feelings.

Naci Kaplan
President of BESD-BIR

Prof. Dr. Necmettin CEYLAN
Chairman of the Congress

ORGANISATION & COMMITTEES

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Chairman of the Organising Committee

Dr. Sait Koca (Besd-Bir)

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6th INTERNATIONAL POULTRY MEAT CONGRESS

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OPENING SPEECHES

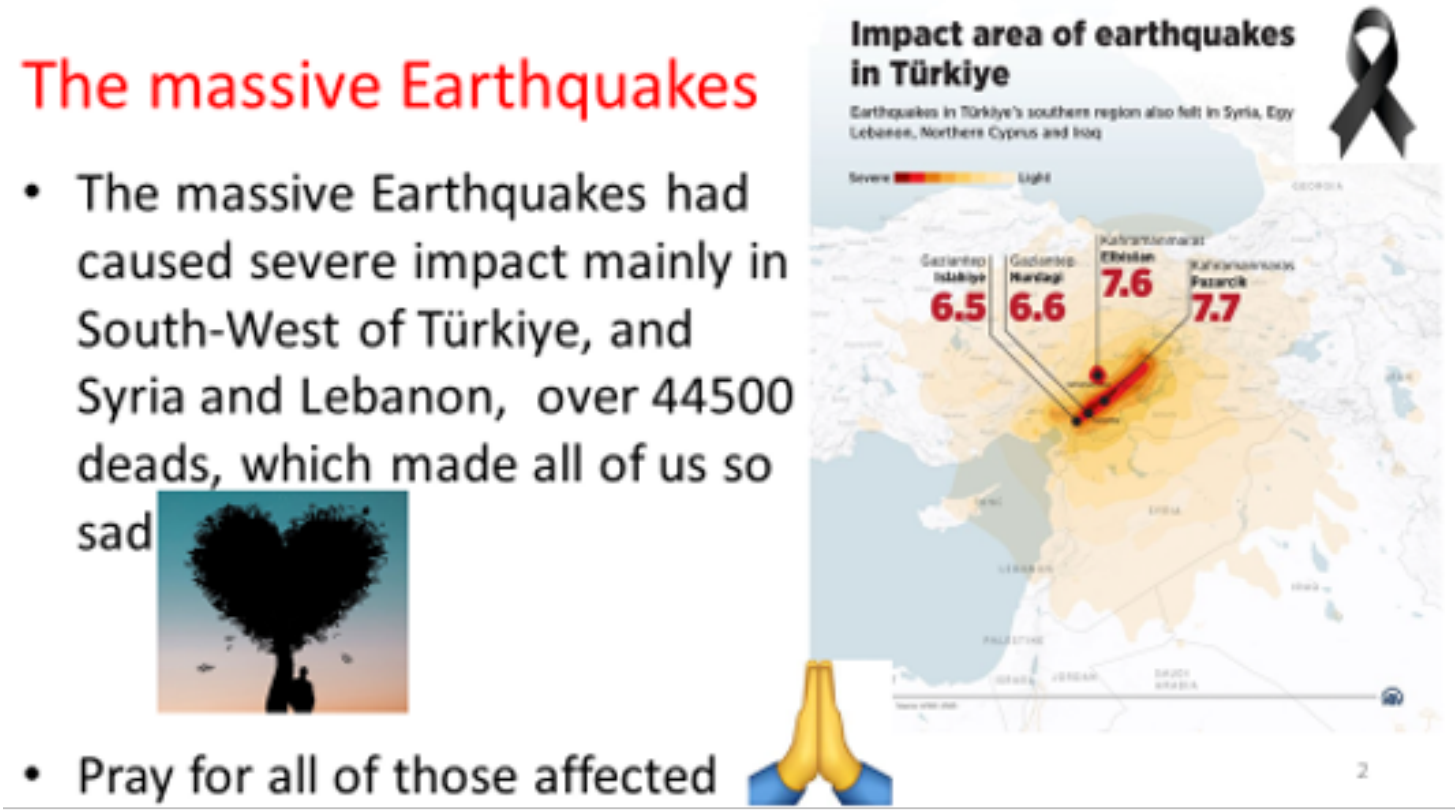
OS[01] International Poultry Meat Congress, Sustainability of Poultry Meat Production and Future Outlook

Necmettin Ceylan

Ankara University, Faculty of Agriculture, Animal Science, 06110 Dıskapı, Ankara, Türkiye

Introduction

Good morning, Dear General Directorate of Food and Cntrol, Dear Department Heads, Managers and all public officials, ear Presidents of Non-Governmental Organizations, Dear Scientists, Dear Representatives and Stakeholders of the Poultry Meat Industry, Dear Members of the Press, Dear Students Welcome to the 6th International Poultry Meat Congress (UBEK-6), the world of science and technology of the poultry meat industry, I greet you all with respect and love. While we are experiencing the excitement and happiness of meeting you again at our 6th congress, which we had to postpone for 2 years due to the covid 19 pandemic in 2021, we are aware of the disaster that occurred in Kahramanmaraş on February 6, affecting Syria and Lebanon and causing the death of more than 50,000 people (Slide 1).) I feel his pain deeply, I wish God's mercy on the dead and a speedy recovery to the injured. My condolences to our entire nation and get well soon. I would like to thank our poultry industry, which has been with the people of the region since the first day. BESD-BİR president Mr. Naci Kaplan will soon give you information about the earthquake-related plans of our congress.



Slide 1.

We were able to hold the first of our congress, which BESD-BİR traditionally decided to organize every 2 years, in May 2011, following a short preparation period under the leadership of Zuhal Daştan, BESD-BİR president at the time, and Dr.

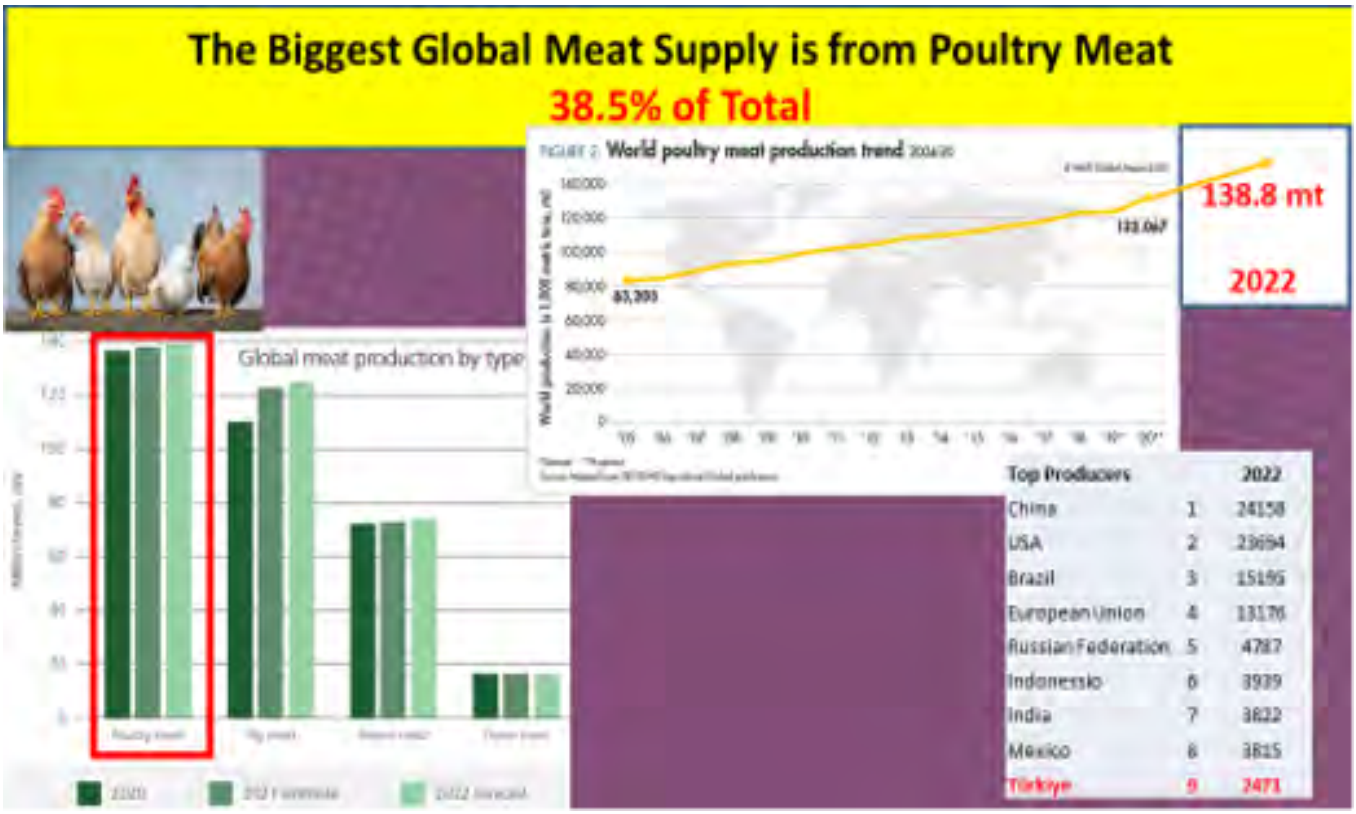
Sait Koca, member of the board of directors. Just as a reflection of the success of the poultry meat industry in Turkey and the world, we have made important progress in our congress and have come to this day. White meat production is of great importance for people to have a healthy, balanced and adequate diet. Turkey's poultry meat sector has always been hand in hand with science and technology while producing. This is the fundamental basis of today's success and healthy production. One of the important goals of all activities carried out by BESD-BİR with this understanding is to become an international brand.

As a matter of fact, our congress has been accepted into the CAB abstract database and started to be scanned with its continuity, paper applications, number of foreign participants, and qualified content. You can also see the numerical breakdown of the intense interest you have shown in our 6th congress (Slide 2). I believe that our congress will achieve greater success in the coming years and will make significant contributions to the stronger integration of the Turkish poultry meat industry with the world.

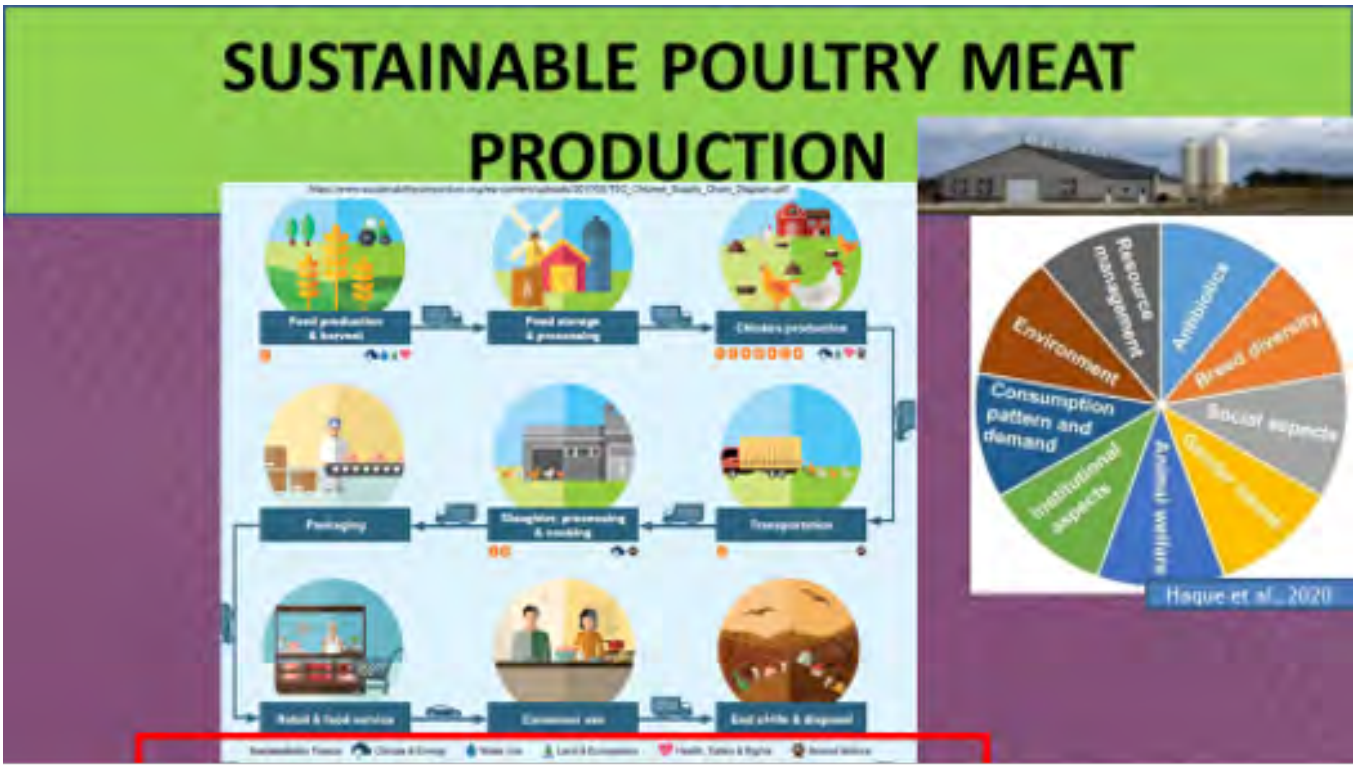
Slide 2. International Poultry Meat Congress in numbers from the 1st to the 6th Congress

As all stakeholders of the sector, we fulfill a sacred duty together. Food production needs to increase steadily in order to ensure that the world population, which is currently 8 billion and is expected to be 9.8 billion in 2050, can be fed healthy, balanced and most importantly adequately, and to prevent hunger.

The chicken meat industry has made the biggest contribution in this regard over the past years and will continue to contribute. As a matter of fact, poultry meat production has become the most produced meat in the world with a rate of 36.5% since 2015, and today the difference has widened and increased to 38.5% (Slide 3). During this period, chicken meat production in Turkey has increased significantly and its growth has reached 37% in the last 9 years. This increase was also reflected in exports and had a major share in the food security of the northern neighbor countries. However, the increasing world population, increasing greenhouse gas emissions and global warming due to industrial and agricultural activities show that we need to pay attention to very different points when producing products. In this context, the need to quickly analyze the methods, threats and risks related to our production and make preparations has emerged. We can also describe this situation as sustainability (Slide 4)



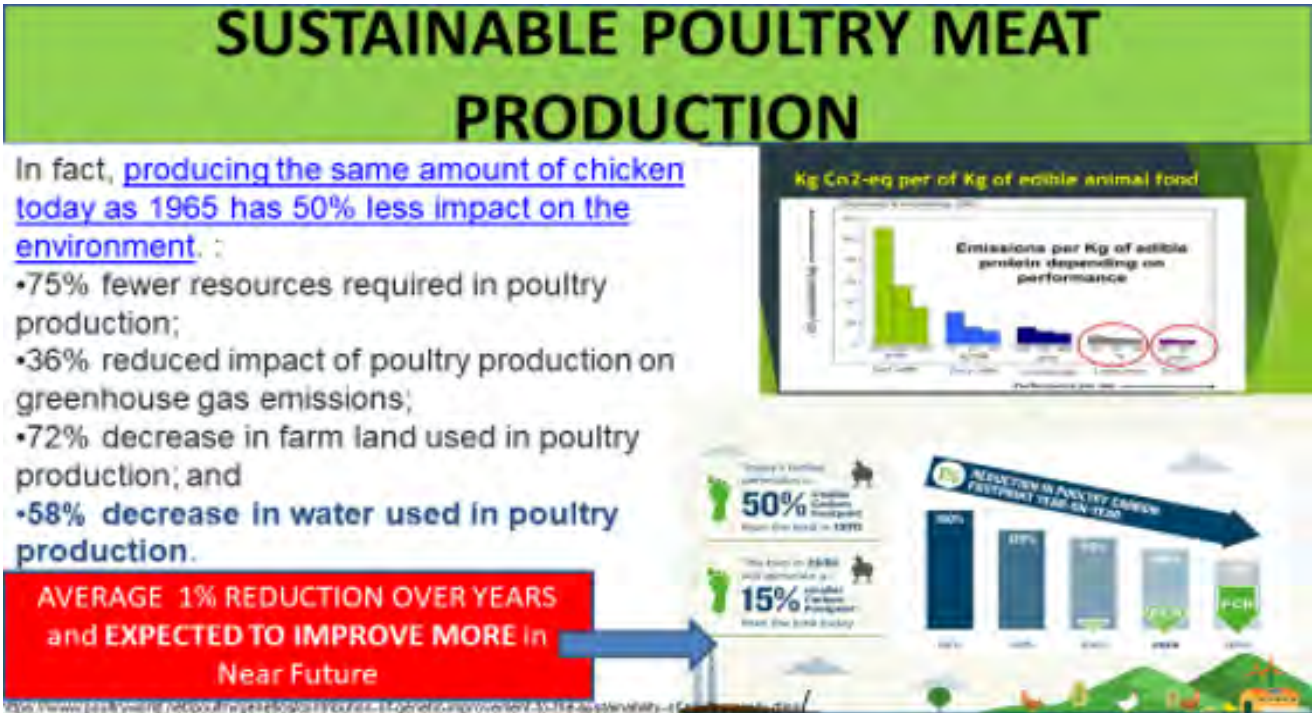
Slide 3. Chicken meat production by years and important producing countries



Slide 4. Sustainability,

Although sustainability is not a new concept, it has become an important issue that we need to focus more on and focus on in production processes in the future. Therefore, we planned to highlight the issue of sustainability and raise our awareness at UBEK-6. Sustainability covers many factors in the production process. Among these, we observe that environmental protection, energy, resource use efficiency, waste management and animal welfare issues come to the fore today, especially due to global warming, and in parallel, issues such as meat quality, consumer demands, national and commercial regulations, and epidemic diseases are also included.

Poultry meat production is actually one of the most successful sectors in terms of sustainability and has made great progress in this field over the years. Compared to 60 years ago, it has managed to save 75% in resource use, 36% in greenhouse gas emissions, 72% in land use and 58% in water use (Slide 5).

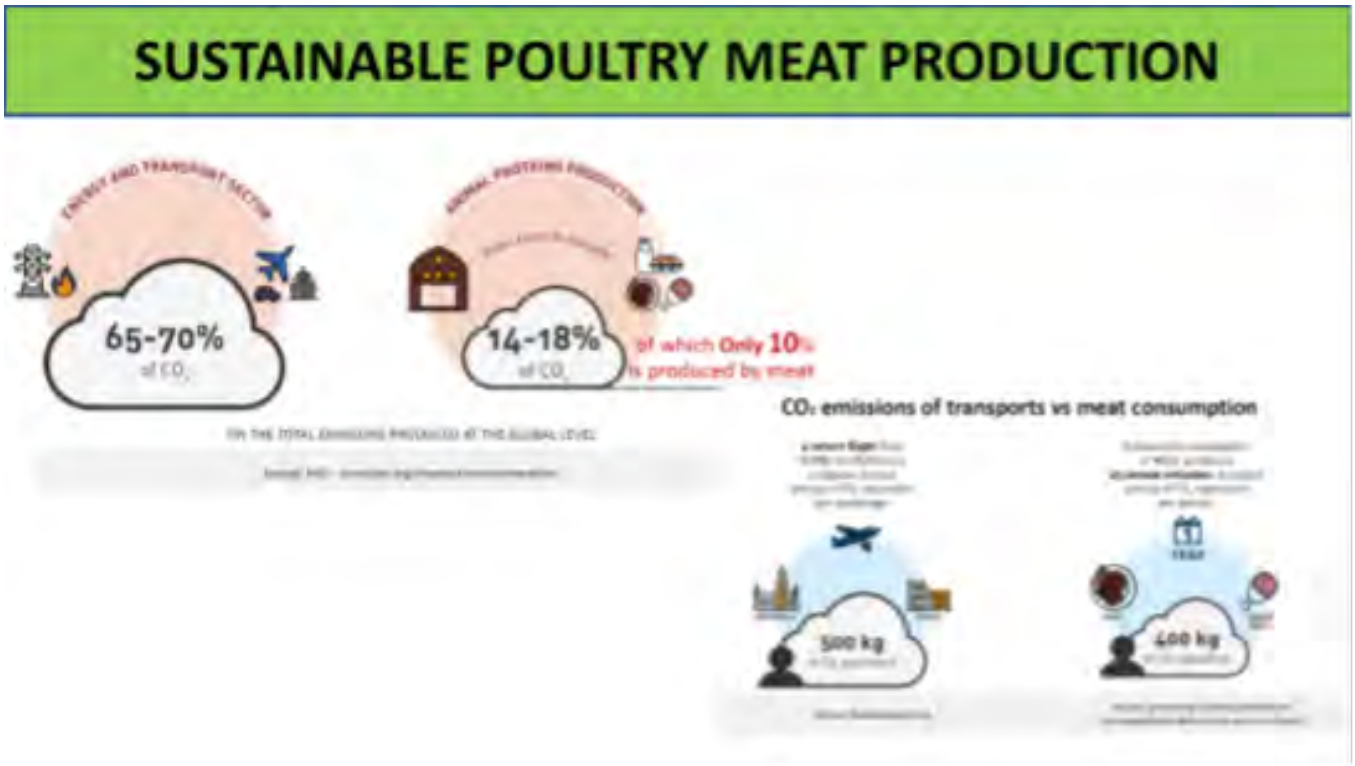


Slide 5. Sustainability and Chicken meat production

The share of food production in total greenhouse gas emissions is 26%, and only 6% of this comes from poultry meat production. However, we will need different methods and practices to reduce the carbon footprint for each stage of the production process. In fact, the sector has taken important steps in this direction. As an example, I would like to show you a short video on the BESD-BİR website, which I think many of you have not watched, to explain the sensitivity and current situation of the sector (Slide 6).

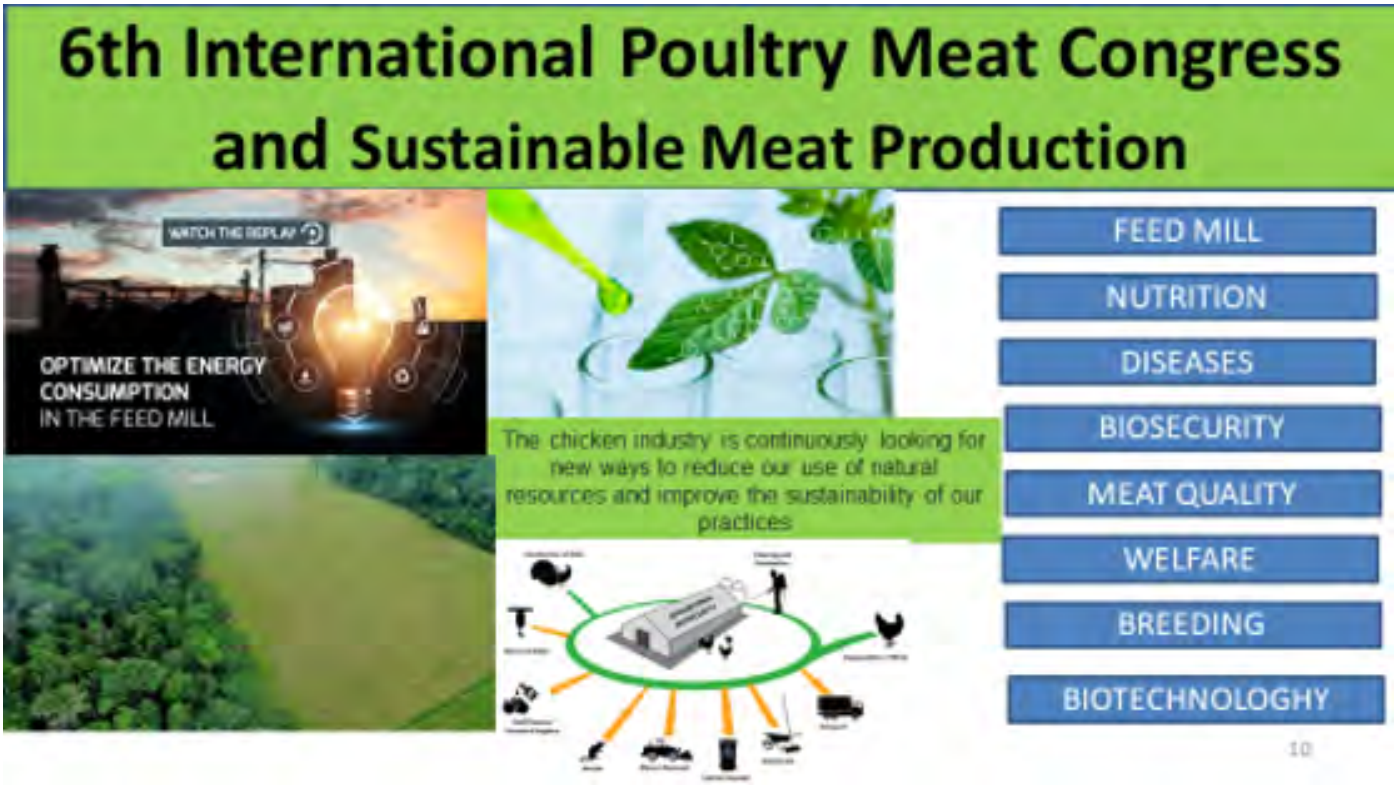
Slide 6. Sustainability and applications in chicken meat production

Certain segments of society, perhaps deliberately or because they do not know the qualities of our sector, have negative thoughts about animal husbandry and meat and milk production, as well as being negative and anti-meat through the media. In such cases, it would be useful for us to be aware of the information that I think will benefit all of us. As you can see, the share of animal protein production is 14-18% of the total greenhouse gas production, and meat production is only 10%. Especially when we look at the carbon footprint, for example, when one person flies from Rome to Brussels, 500 kg of CO2 equivalent emissions occur, while one person's annual meat consumption is only 400 kg of CO2 equivalent (Slide 7).



Slide 7. Chicken meat production air travel greenhouse gas emissions comparison

I would also like to place special emphasis on a few issues in terms of sustainable production. In broiler production, the feed factory accounts for 50-58% of the total energy use. We should pay more attention to this data (Slide 8). With the increasing production every year, the parallel sector's need for soybeans increases day by day, and more soybean production may create a situation that is detrimental to the forest. In this case, we have to improve the use of alternative feed sources and feed utilization efficiency. It is important that breeding companies carry out studies that can better evaluate soy-free diets, and we hope that these studies will make a great contribution to us in the near future. Likewise, we need to evaluate biotechnology in this context. In our congress, we will discuss these issues with the participation of many scientists who are experts in their fields. I hope that we will start today and carry out an activity together for 3 days that will produce beneficial results for our sector.



Slide 8. Important issues in terms of sustainability in chicken meat production

I would like to thank our esteemed invited speakers who attended our congress from abroad and from home, who will enlighten us with their valuable information and share their knowledge and experiences, and all the valuable academicians and researchers who supported our congress by sending papers and made it possible to create this scientific environment, for their contributions.

I would like to express my great gratitude and appreciation to all our sponsors, especially our main sponsor Aviagen, for their invaluable support and presence. In order to achieve a high-level congress worthy of our country's white meat industry, BESD-BİR board of directors, who spare nothing for the best and better in terms of science, provide great support to the scientific world of our country by providing oral presentation support to academics and free participation scholarships to 60 students, and I would also like to express my thanks and gratitude to Mr. President Naci Kaplan, Dr. Sait Koca and Zuhal Daştan, who shaped and initiated the idea of holding the congress traditionally every two years, and of course, BESD-BİR, the owner of the congress, and the congress organizing group. Finally, I would like to thank the valuable members of the press and all of you who attended our congress and accompanied us for being here, and I wish you a successful congress.

OS02 6th International Poultry Meat Congress and BESD-BİR

Naci Kaplan

President of BESD-BİR
Association of Poultry Meat Producers and Breeders (BESD-BİR), Ankara, Türkiye

Dear General Managers, Department Heads and Officers of Our Ministries,

Esteemed Representatives of Our Universities, Presidents and Representatives of Non-Governmental Organizations, Esteemed Guests from All Around the World, Honorable Speakers, Esteemed Members of Our Association, All the Stakeholders of Our Industry, Press Members, Ladies and Gentlemen,

I would have loved to welcome you with great enthusiasm with the pride and satisfaction of organizing the 6th International Poultry Meat Congress on the 100th anniversary of our Republic and 30th anniversary of our Association when we had to postpone the Congress for two years due to COVID-19 pandemic... However, we are deeply sorry about the earthquake that hit Kahramanmaraş on February 6 and deeply affected another 10 provinces... May all those we have lost in this disaster rest in peace. In your presence, I would like to repeat my condolences to the relatives of the lives we have lost. The companies within our industry mobilized as of the second day to provide food aid and other aids that were the primary deficits in the region. We tried to reach as many people as we could; we did the best we could and will continue to do so. Our hearts and minds are with the earthquake victims; we shall never forget and let forget. We will treat our wounds together.

We must also not disrupt our works to maintain such continuity. We gathered with our Congress Organization Committee right after the disaster. We decided not to postpone this congress that created scientific data for the Turkish poultry meat industry and that had been in preparation for almost two years. Because we had to work and produce more to help our country rise on her feet once again. As BESD-BIR (Association of Poultry Meat Producers and Breeders), we acted in light of science since our establishment, and we use scientific data to develop and advance our industry. We think that it is very important to provide contributions to our industry while producing information besides our products. We would like to offer many thanks to you for not leaving us alone during this sensitive and challenging situation.

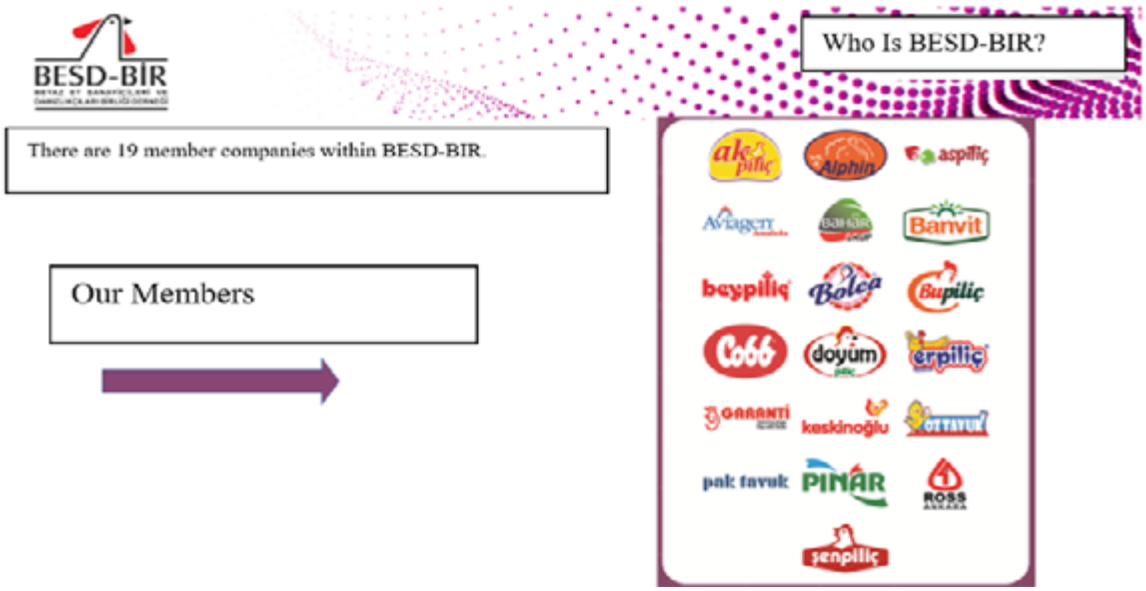
We have taken some decisions about our congress and would like to share them with you. We used to organize a Gala Night at every congress as a celebration to relax, but we excluded that event from our congress to show respect for over 45,000 lives we have lost to the disaster. We decided to use the funds that we would have to spend for the gala night to provide aid to the earthquake victims on behalf of you. I would like to mention that we will continue to inform you about our aids to the earthquake victims.

We will carry out the award ceremony for our valuable sponsors who support us and stand by us after the opening speeches and before the exhibition opening and poster visits. This year, we are organizing the 6th International Poultry Meat Congress, which is one of the most important in its field. The interest in our congress is high this year as well. There are 800 participants not including the spouses and children. We are hosting guests from 32 different countries.

Association of Poultry Meat Producers and Breeders represents the Turkish poultry meat industry at the highest level. We can summarize the purpose of our Association as: “carrying out works to develop the Turkish poultry meat industry, create value that will contribute to the enterprises, represent the industry the best possible way, and ensure necessary communication within the public-industry-university relationships.”

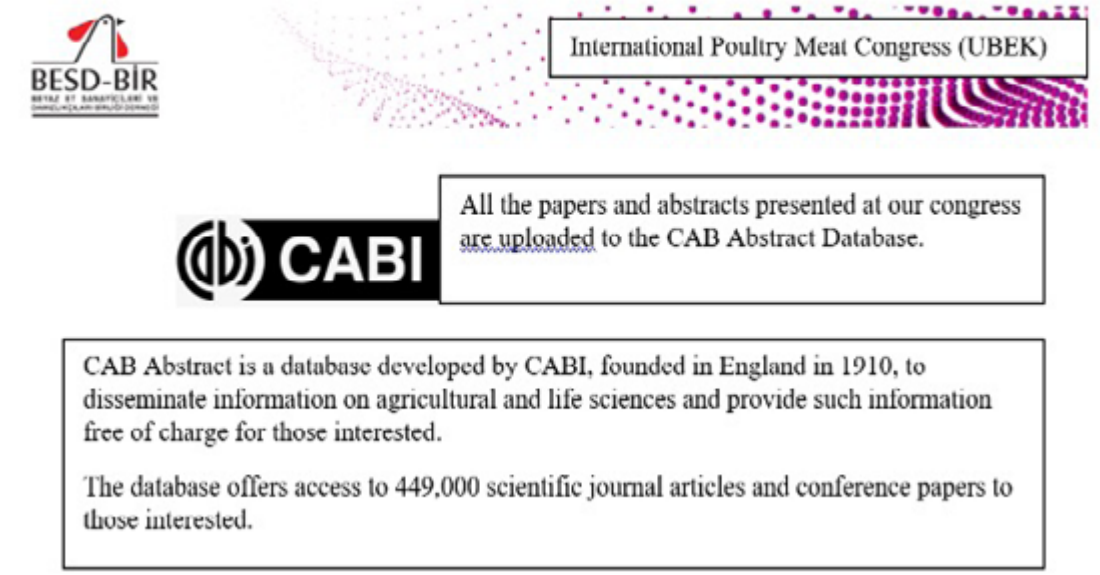
There are 19 companies within BESD-BIR (Slide 1). Education is the first of our priorities as the “Turkish Poultry Meat

Industry". In this congress, we will update our knowledge, catch up with the latest developments, discuss the issues and find solutions together. The first-day sessions of our congress will be in this room and the second- and third-day sessions will be in three different rooms that will be created in this area. There will be 19 sessions in the 6th International Poultry Meat Congress. There are also two satellite symposia in the congress program. 60 students who receive education in different faculties related to our industry were provided scholarship to participate in our congress.



Slide 1. BESD-BIR Member Companies.

This year, our congress has a mobile app. UBEK 2023. When you download the app from Google Play Store and Apple Store to your mobile phone, you can monitor the processes related to the congress. CAB Abstract is a database developed by CABI, founded in England in 1910, to disseminate information on agricultural and life sciences and provide such information free of charge for those interested. All the papers and abstracts presented at our congress are uploaded to the CAB Abstract Database (Slide 2). This is important in terms of worldwide recognition and acknowledgement of our congress.



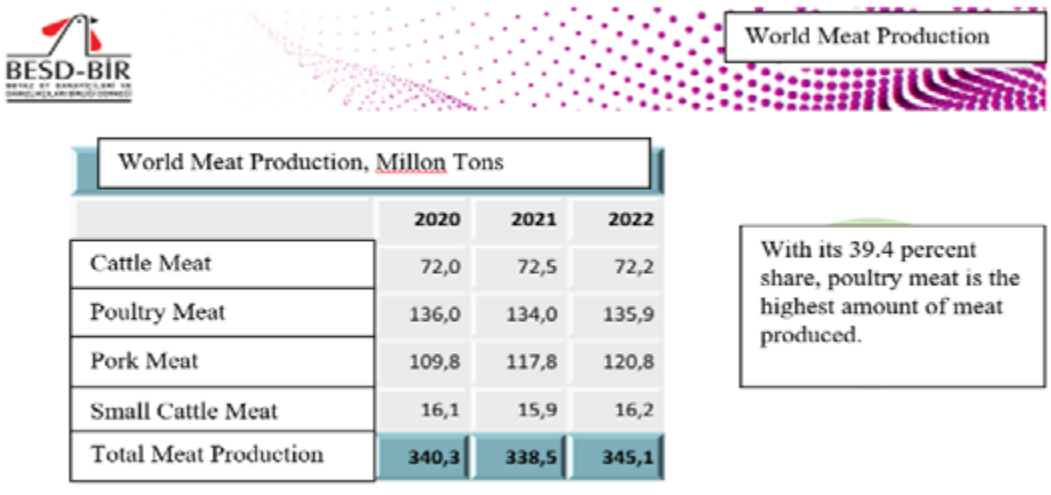
Slide 2. UBEK-6 International Indexing

It has been 30 years since BESD-BIR was founded in 1993. Turkish poultry meat industry's today was created by constant development. The material and moral support of our Ministry of Agriculture and Association members in our success is undeniable. Our stakeholders in the industry have also contributed greatly. I owe everyone who supported us a debt of gratitude. I fully believe that we will carry out many breakthroughs and works in unity in the future. For the past 17 years as one of the five founding members and representative of the Turkish poultry meat industry, our Association has been an active member of the International Poultry Council, an organization where 30 countries that carry out 95% of the world's poultry trade are members (Slide 3). There are 30 full members of the International Poultry Council. There are also 54 associate members. Mr. Nikolo Cinotti, the General Secretary of this Council, is also with us today. I pay my respects and offer my thanks to Mr. Cinotti for taking the time to join us today.

Slide 3. BESD-BIR and IPC Membership

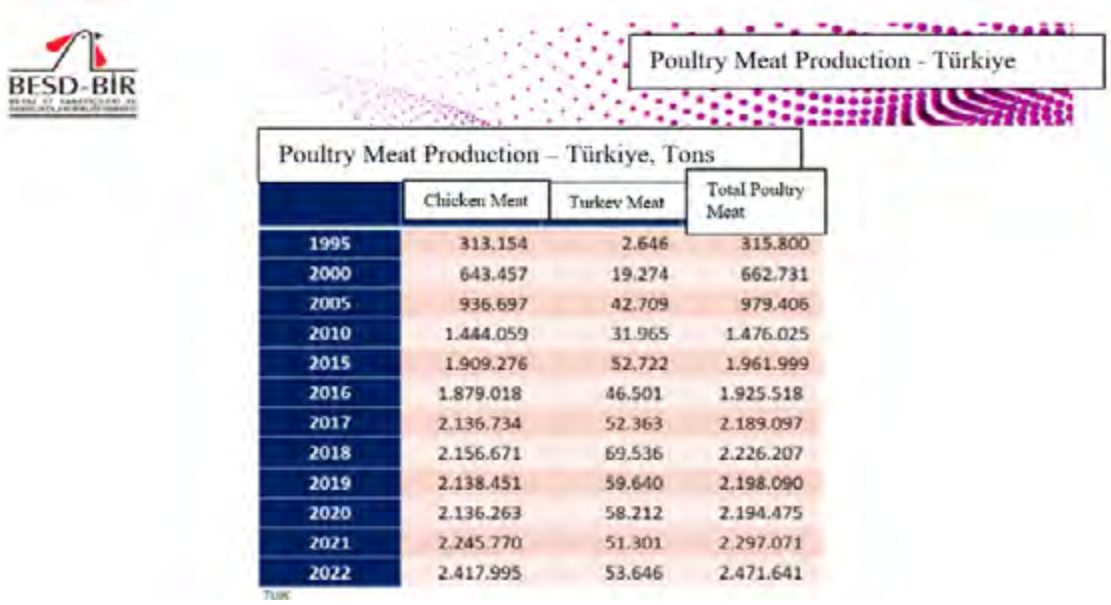
According to the Food and Agriculture Organization (FAO), the global hunger numbers rose to 828 million in 2021. FAO's report on food production indicates that the number of countries that need outside food aids rose to 45. According to the Report on Food Security and Nutrition, 3.1 billion people, about 40 percent of the world population, cannot reach healthy nutrition.

In 2022, total meat production in the world was 345.1 million tons, and poultry meat production had the largest share with 39.4 percent and 135.9 million tons (Slide 4). United States of America, China and Brazil are leading the world chicken production by a wide margin. According to the USDA data, Türkiye ranks 8th.



Slide 4. World Meat Production

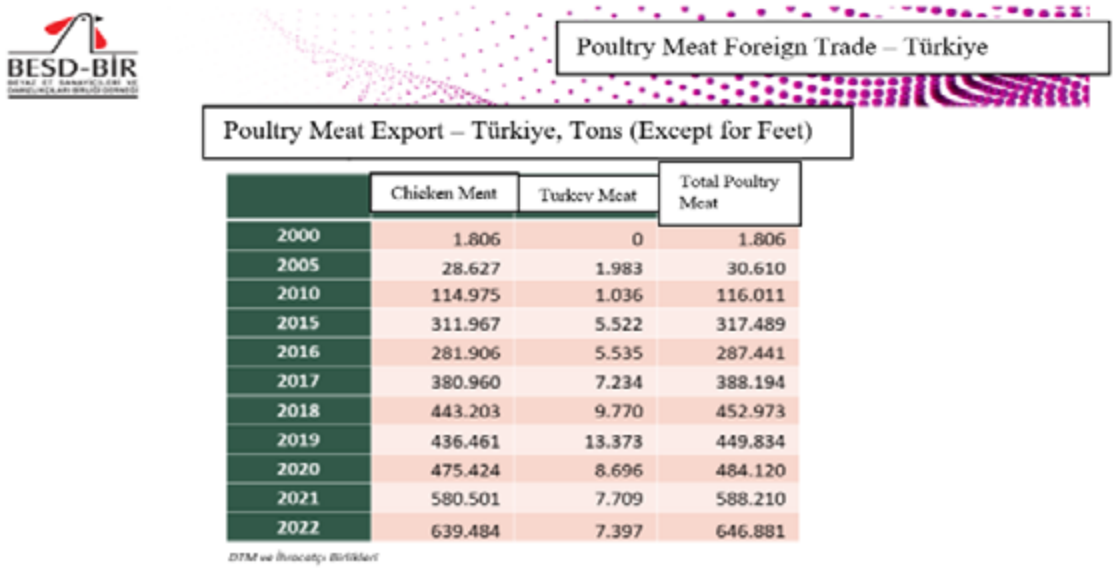
We are producing by implementing the national and international quality standards at the highest level and conforming to the principles of food security meticulously. The poultry meat industry continues to develop rapidly, dedicating itself to the sustainable and environment friendly production applications which are the two of the most important issues of the new world order. In 1995, poultry meat production in Türkiye was 316 thousand tons and per capita consumption was 5 kilograms. Today, we reached 2.470 million tons in poultry meat production. Chicken meat production is 2.420 million tons, and turkey meat production is 54 thousand tons (Slide 5).



	Chicken Meat	Turkey Meat	Total Poultry Meat
1995	313.154	2.646	315.800
2000	643.457	19.274	662.731
2005	936.697	42.709	979.406
2010	1.444.059	31.965	1.476.025
2015	1.909.276	52.722	1.961.999
2016	1.879.018	46.501	1.925.518
2017	2.136.734	52.363	2.189.097
2018	2.156.671	69.536	2.226.207
2019	2.138.451	59.640	2.198.090
2020	2.136.263	58.212	2.194.475
2021	2.245.770	51.301	2.297.071
2022	2.417.995	53.646	2.471.641

Slide 5. Poultry Meat Production in Türkiye

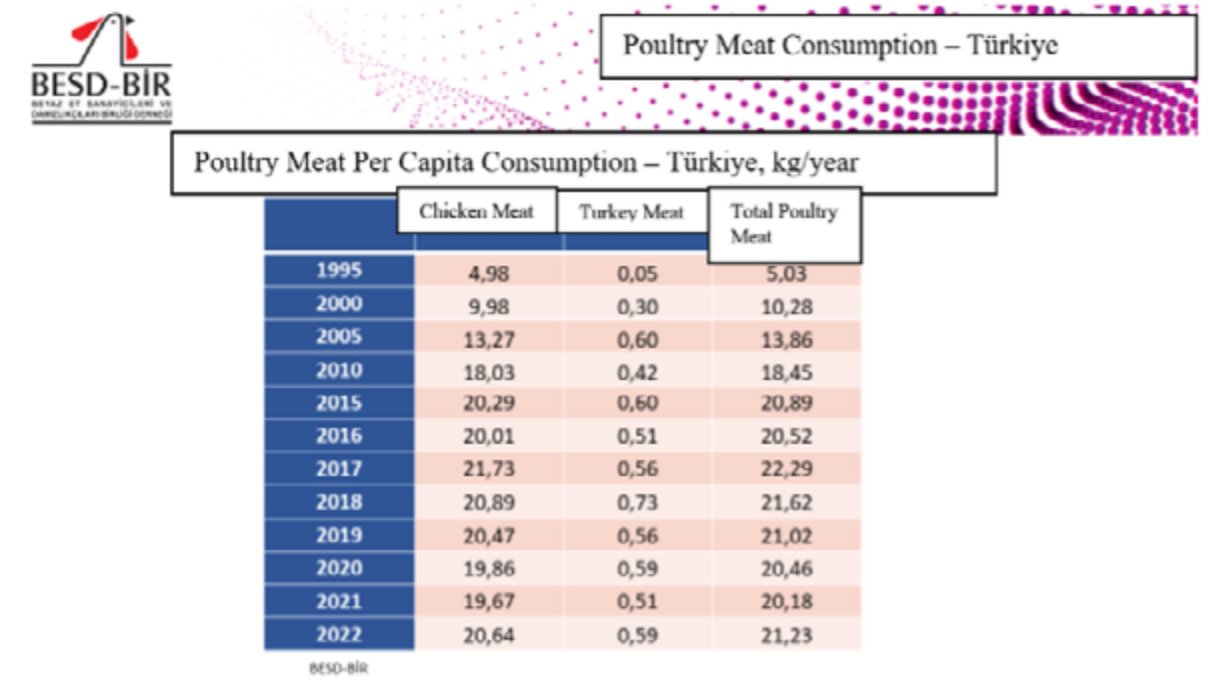
Poultry meat exports of Türkiye constantly increased and reached 646,880 tons except feet in 2022 (Slide 6). Chicken feet export is around 50 thousand tons. We value export greatly. While reaching such numbers, the number of export countries also increased constantly, and 97 countries received our exports in 2022. The country we export the most is Iraq. We continue our cooperation with Ministries of Agriculture and Trade to create new markets and grow our current markets. When we examine the countries that rank high in the annual chicken meat consumption per capita in the world, we see that the top 10 countries average 38-58 kg. When we consider that the world average is around 20 kg, we see that there are still many ways to go for other countries to reach top 10.



	Chicken Meat	Turkey Meat	Total Poultry Meat
2000	1.806	0	1.806
2005	28.627	1.983	30.610
2010	114.975	1.036	116.011
2015	311.967	5.522	317.489
2016	281.906	5.535	287.441
2017	380.960	7.234	388.194
2018	443.203	9.770	452.973
2019	436.461	13.373	449.834
2020	475.424	8.696	484.120
2021	580.501	7.709	588.210
2022	639.484	7.397	646.881

Slide 6. Poultry Meat Export of Türkiye

Per capita chicken meat consumption in Türkiye is 20.6 kg annually (Slide 7). When we consider that pork meat is not consumed by the Muslim population of Türkiye, we can say that our success story of 4.5 times increase in the past 30 years is lacking. Chicken meat consumption levels remain the same for the past four years. Unfortunately, unscientific claims regarding our industry created misperceptions in the public. We need our Ministry's support regarding that. To correct the misleading information in public regarding the poultry meat industry, we are sure that the public service ads by our Ministry will contribute greatly for the recognition of the importance of poultry meat in a balanced and healthy nutrition.



	Chicken Meat	Turkey Meat	Total Poultry Meat
1995	4,98	0,05	5,03
2000	9,98	0,30	10,28
2005	13,27	0,60	13,86
2010	18,03	0,42	18,45
2015	20,29	0,60	20,89
2016	20,01	0,51	20,52
2017	21,73	0,56	22,29
2018	20,89	0,73	21,62
2019	20,47	0,56	21,02
2020	19,86	0,59	20,46
2021	19,67	0,51	20,18
2022	20,64	0,59	21,23

BESD-BİR

Slide 7. Poultry Meat Consumption per Person in Türkiye

With its 5.5 billon Dollar revenue, the poultry meat industry in Türkiye has created a structure that provides employment for 3 million people, directly and indirectly. For that, the poultry meat industry has always followed the developments in the world in light of science, applied all the modern and advanced technologies, utilized earnings and capital as investment, created employment in rural areas and attached great importance to education.

Today, we take pride in producing safe food that reached international standards in quality and healthy production and that is fully recorded. Our industry assumed a very important role for filling in the animal protein deficit of the public, and always acts with such sense of mission.

Use of antibiotics as growth factor is banned in Türkiye. Under the leadership of BESD-BIR, our works regarding the decrease of the use of antimicrobials by increasing the biosecurity measures in 2016 made great progress. Almost all the legislation in Türkiye regarding our industry is in conformity with the EU legislation.

The number of certified biotechnology products is 541 in the world, 280 in the European Union and only 36 in Türkiye. This causes our costs to increase and makes it challenging for us to be competitive in exports. Our greatest wish is to have the Biosecurity Law and other regulations that regulate the biotechnology products conform with the European Union legislation.

We take pride in our industry, all of its employees and breeders and all our stakeholders who make healthy and quality production by meeting the national and international standards at the highest level for the past 30 years without compromising their purposes under our Association.

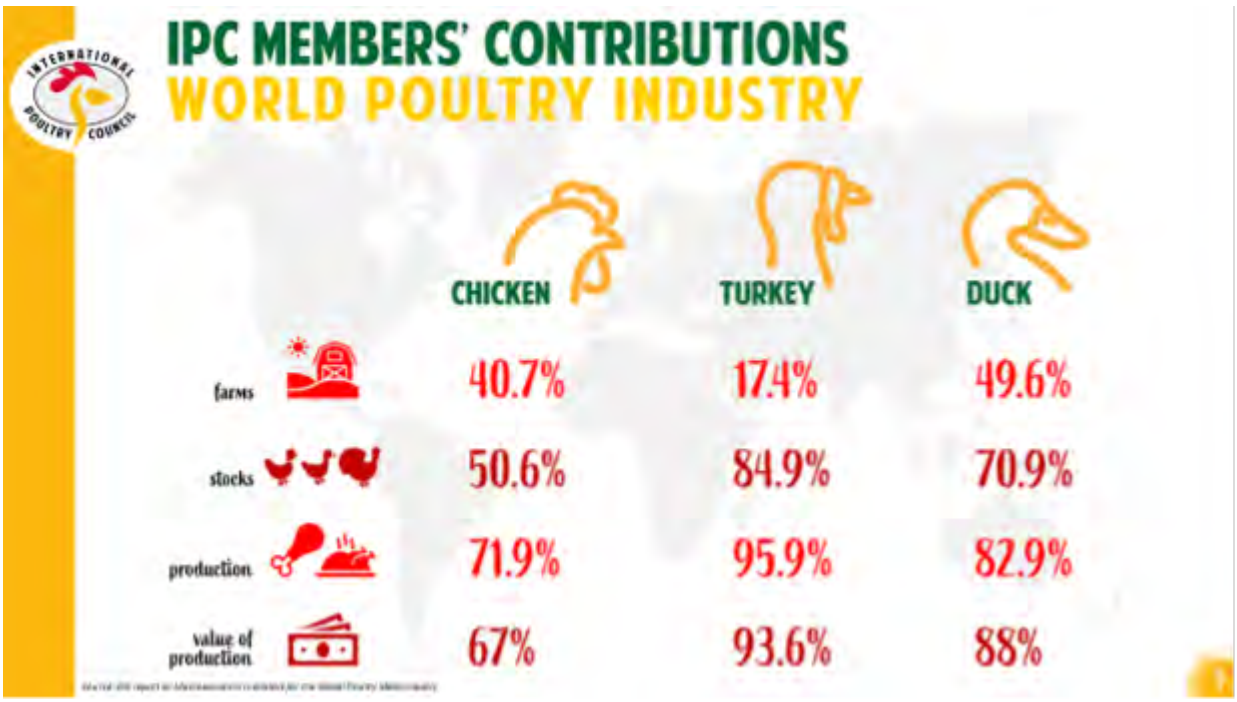
We would like to thank to those who contributed to the organization of this congress and especially to the Chair of our Congress Prof. Necmettin Ceylan, our Board Member and Congress Organization Committee President Dr. Sait Koca, members of the organization committee, members of the scientific board, scientists who contributed to our Congress with

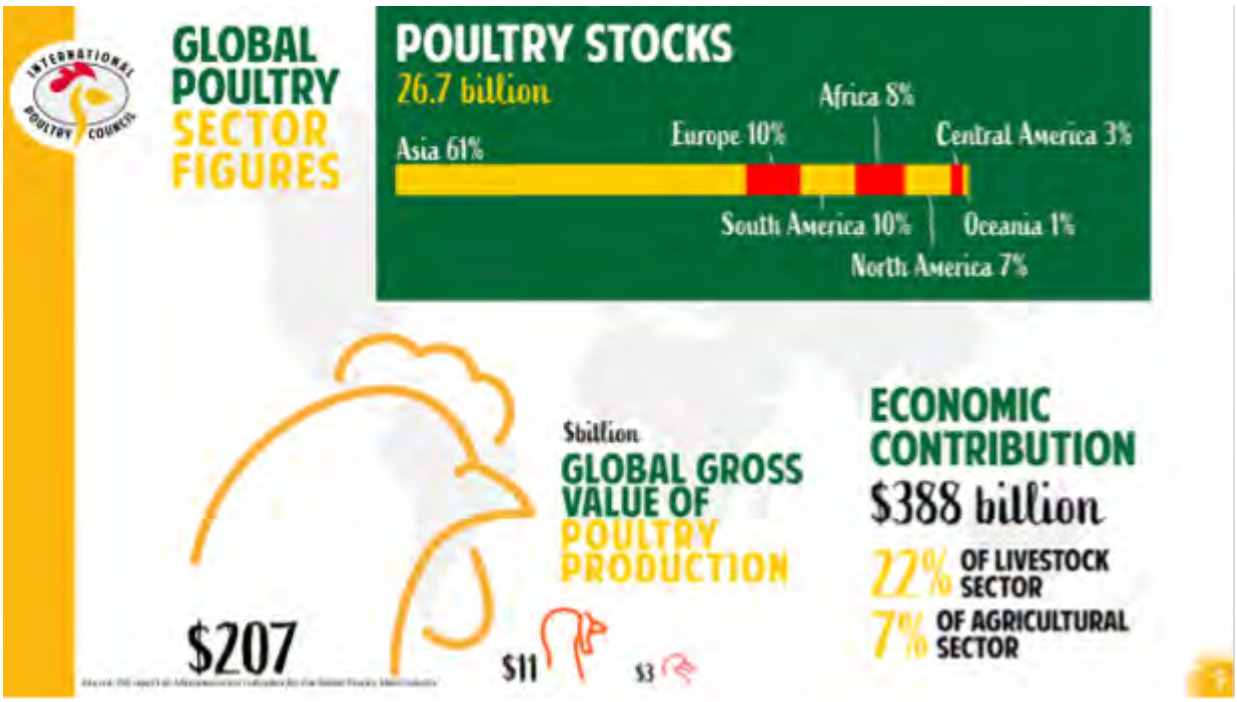
their presentations and posters, our session chairs, Papyon Organizasyon who organized the Congress, all the companies who supported us as sponsors and by purchasing booths, BESD-BIR employees, the employees of Titanik De Lüks Golf Hotel who hosted us, and in short, to all who contributed to the congress and to you valuable participants.

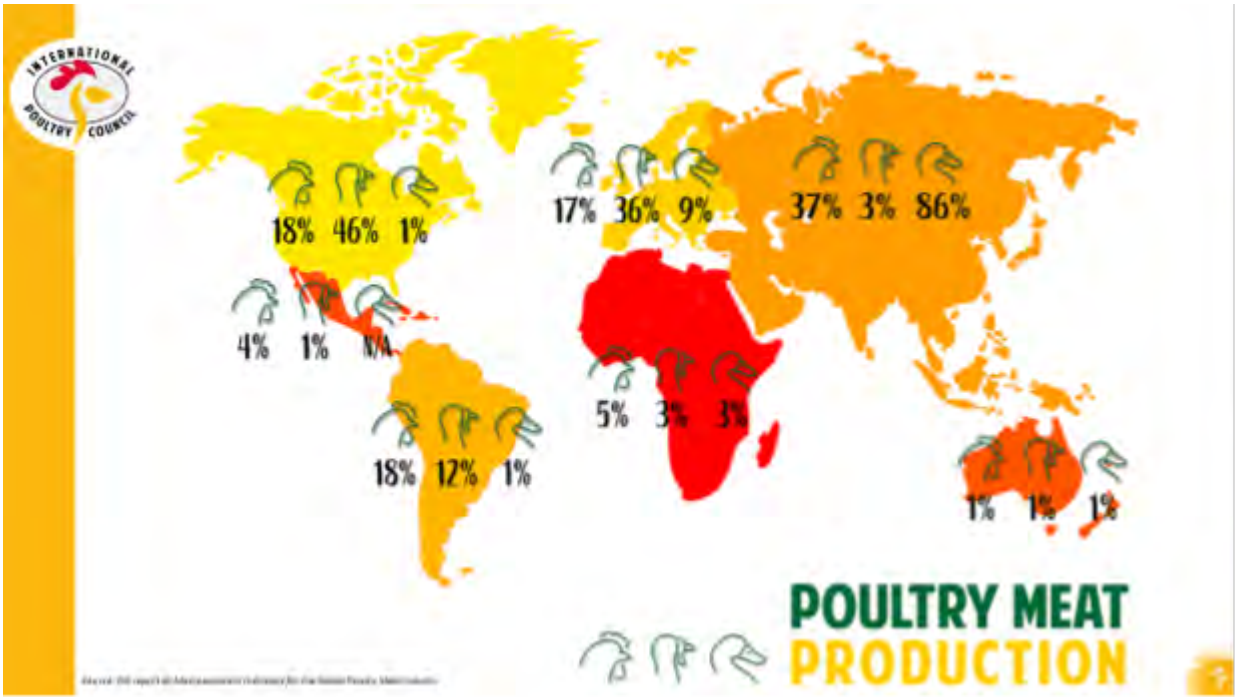
I pay all of you my respects, wishing a successful and efficient congress, and once again offer my condolences to all our citizens who lost their lives in the earthquake that hit 11 provinces including Kahramanmaraş and their relatives and wish a swift recovery to all our people and especially those who try to continue their lives in the region. I salute you, wishing a successful and efficient congress.

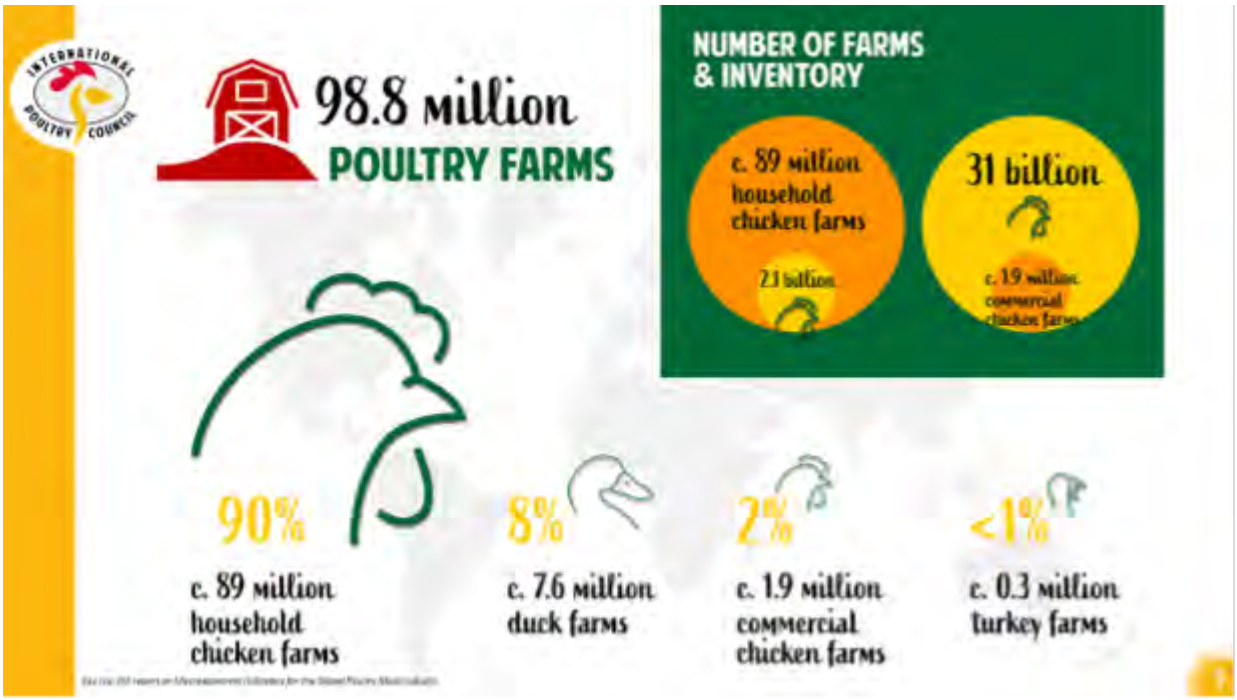
OS[03] Global Poultry Meat Industry-Scenario and Challanges

Nicolò Cinotti
Secretary General of International Poultry Council, Tucker, GA 30084, USA


IPC MEMBERS' CONTRIBUTIONS
WORLD POULTRY INDUSTRY
CHICKEN
TURKEY
DUCK
farms
40.7%
17.4%
49.6%
stocks
50.6%
84.9%
70.9%
production
71.9%
95.9%
82.9%
value of production
67%
93.6%
88%


GLOBAL POULTRY SECTOR FIGURES
POULTRY STOCKS
26.7 billion
Asia 61%
Europe 10%
Africa 8%
Central America 3%
South America 10%
Oceania 1%
North America 7%
$207
$11
$3
$billion
GLOBAL GROSS VALUE OF POULTRY PRODUCTION
ECONOMIC CONTRIBUTION
$388 billion
22% OF LIVESTOCK SECTOR
7% OF AGRICULTURAL SECTOR


INTERNATIONAL POULTRY COUNCIL
18% 46% 1%
4% 1% N/A
18% 12% 1%
17% 36% 9%
5% 3% 3%
37% 3% 86%
1% 1% 1%
POULTRY MEAT PRODUCTION


INTERNATIONAL POULTRY COUNCIL
98.8 million
POULTRY FARMS
NUMBER OF FARMS & INVENTORY
c. 89 million household chicken farms
2.1 billion
31 billion
c. 1.9 million commercial chicken farms
90%
c. 89 million household chicken farms
8%
c. 7.6 million duck farms
2%
c. 1.9 million commercial chicken farms
<1%
c. 0.3 million turkey farms

,

Production and export - 2022

Country	.000 tonns
US	20.712
Brazil	14.750
China	14.300
EU	10.910
Russia	4.650
Mexico	3.900
Thailand	3.340
Argentina	2.300
Turkey	2.230

Country	.000 tonns
Brazil	4.325
US	3.403
EU	1.790
Thailand	960
Turkey	515
China	460

Trade - 2021

TRADE FLOW VOLUMES AND MARKET SHARE

Select Trade Flow: Export

Select Year: 2014 2015 2016 2017 2018 2019 2020 2021

Select Product:
- Selezione tutti
- Chicken
- Duck and Guinea Fowl
- Duck, Geese and Guinea Fowls
- Ducks
- Guinea

Select Category:
- Selezione tutti
- Cuts and edible offals, Fresh or Chilled
- Cuts and edible offals, Frozen
- Cuts, Fresh or Chilled
- Cuts, Frozen
- Fatty Livers, Fresh or Chilled
- Meat of Domest Fowls
- Meat Preparations (excl. sausages)
- Offal Meats, Fresh or Chilled
- Offal Meats, Frozen
- Poultry Fats
- Salted meat

Reporter Export Volume and Participation in 2021

Reporter	Volume (tons)	%
Brazil	4.682.217	24,09%
USA	4.291.709	22,08%
Poland	1.812.471	9,32%
Netherlands	1.649.479	8,49%
Germany	950.812	4,89%
Turkey	874.883	4,47%
Belgium	600.673	3,09%
China	565.488	2,91%
France	525.731	2,70%
Ukraine	477.598	2,45%
United Kingdom	415.662	2,14%
Spain	373.680	1,92%
Hungary	309.253	1,59%
Italy	301.583	1,55%
Belarus	245.388	1,26%
Denmark	205.484	1,06%
Canada	180.934	0,93%
Totale	19.439.738	100,00%

Partner Export Volume and Participation in 2021

Partner	Volume (tons)	%
China	1.203.501	8,19%
Germany	1.195.257	8,15%
Mexico	1.150.997	5,92%
Netherlands	1.107.694	5,70%
France	782.725	4,03%
Japan	701.789	3,61%
United Kingdom	597.710	3,07%
United Arab Emirates	497.328	2,56%
Belgium	448.052	2,30%
Philippines	444.694	2,28%
South Africa	434.075	2,23%
China, Hong Kong SAR	391.675	2,02%
Saint Lucia	365.552	1,88%
Cuba	358.645	1,84%
Ghana	358.045	1,84%
Iraq	350.697	1,80%
Angola	307.393	1,58%
Totale	19.439.738	100,00%

Focus: Turkiye - 2021

GLOBAL CHICKEN MEAT SUPPLY & DEMAND TABLE

- Turkiye is the 9th global producer and the 5th global exporter
- More than 140 trade partners
- Production and export trends constantly growing in the last 20 years

TRADE FLOW VOLUMES AND MARKET SHARE

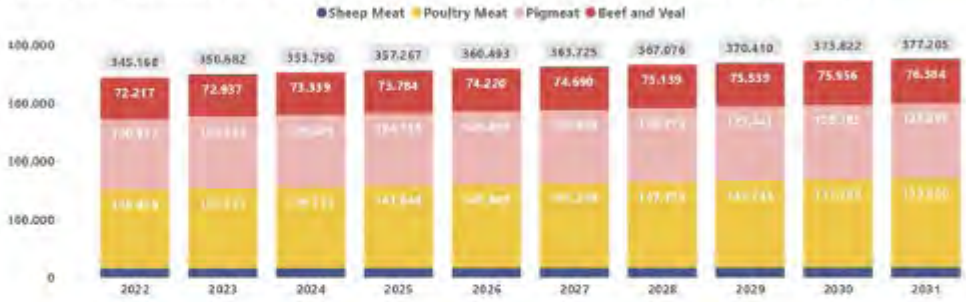
GLOBAL PROJECTIONS (OECD-FAO)

Select Publication Year:
2020
2021
2022

Select Location:
Developed Countries
Developing Countries
OECD
World

Select Criteria:
Consumption
Production

Future World Projections of the Production of All Animal Proteins, Published in 2022 (tonnes cwe or tonnes rtc)



Future World Projections of the Production of All Animal Proteins, Published in 2022 (tonnes cwe or tonnes rtc)

Period	2022		2023		2024		2025		2026		2027		2028		2029		2030		2031	
Protein	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc	cwe	rtc
Beef and Veal	72.217		72.937		73.339		73.784		74.220		74.690		75.139		75.539		75.956		76.384	
Pigmeat	120.822		123.512		124.026		124.758		125.349		125.992		126.713		127.441		128.185		128.895	
Poultry Meat		135.929		137.777		139.715		141.648		143.608		145.748		147.725		149.733		151.788		153.650
Sheep Meat	16.201		16.455		16.670		16.877		17.086		17.295		17.490		17.692		17.893		18.076	

OBS 1: Beef & Veal, Pigmeat and Sheep Meat data expressed in Kilotons of Carcass Weight (kt cwe), Poultry Meat expressed in Kilotons of Ready to Cook (kt rtc).
OBS 2: Carcass Weight to Retail Weight (rtc) conversion factors of 0.7 for Beef & Veal, 0.78 for Pigmeat and 0.88 for both Sheep Meat and Poultry Meat.

GLOBAL PROJECTIONS COMPARISONS (OECD-FAO)

Select Protein:
- Beef and Veal
- Pigmeat
- Poultry Meat
- Sheep Meat

Select Location:
- Developed Countries
- Developing Countries
- OECD
- World

Select Criteria:
- Consumption
- Production

OECD-FAO Production Projections of Poultry Meat in the Next Decade in the World per Year of Publication

Publication Year	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031
2020	132.067	133.301	134.561	136.116	137.686	139.256	140.831	142.436	144.076	145.711		
2021		135.071	137.280	138.413	140.621	142.773	144.858	146.960	149.124	151.307	153.479	
2022			135.829	137.777	139.715	141.848	143.808	145.748	147.725	149.733	151.788	153.850

OECD-FAO Production Projections of Poultry Meat in the Next Decade in the World per Year of Publication

OBS 1: Beef & Veal, Pigmeat and Sheep Meat data expressed in Kilotons of Carcass Weight (kt cwe); Poultry Meat expressed in Kilotons of Ready to Cook (kt rtc)
OBS 2: Carcass Weight to Retail Weight (rtc) conversion factors of 0.7 for Beef & Veal, 0.78 for Pigmeat and 0.88 for both Sheep Meat and Poultry Meat

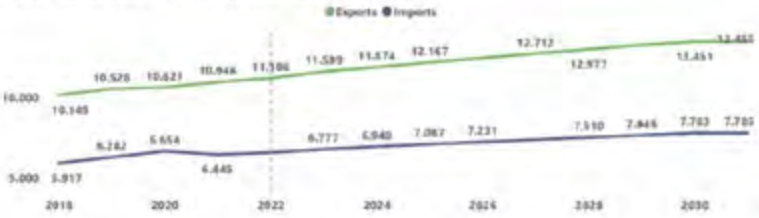
GLOBAL EXPORT AND IMPORT PROJECTIONS (USDA)

Select Publication Year:
2022

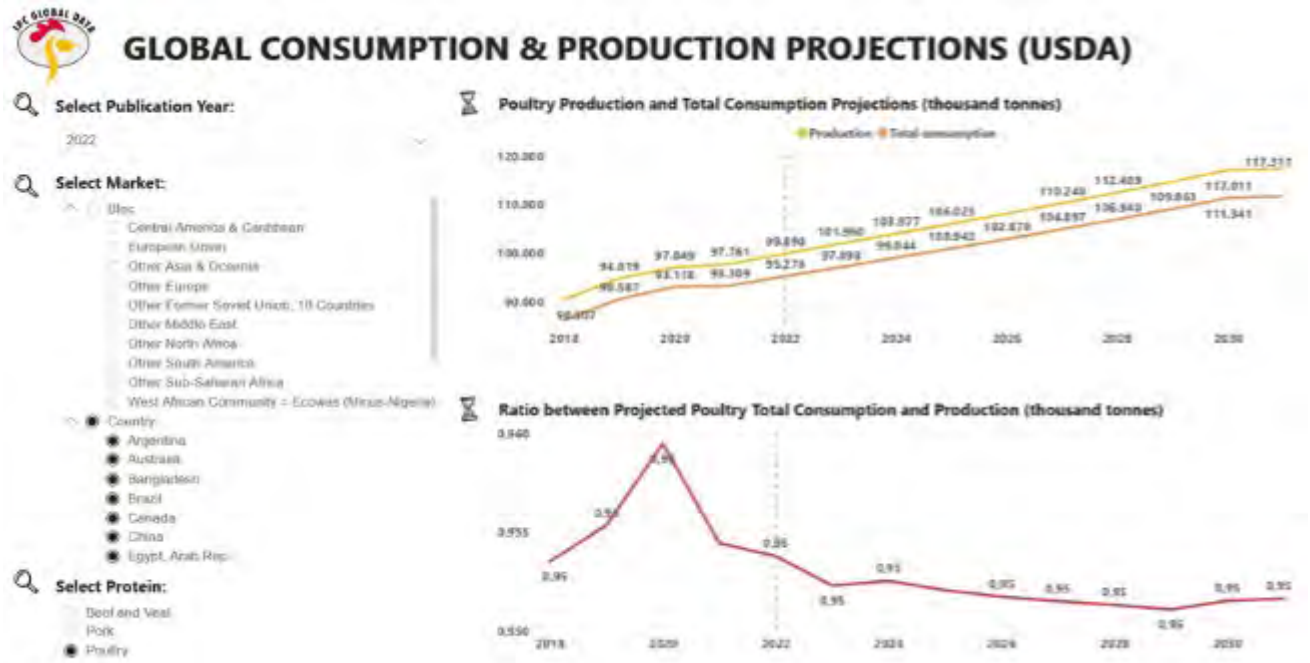
Select Market:
- Rtrc
- Central America & Caribbean
- European Union
- Other Asia & Oceania
- Other Europe
- Other Former Soviet Union, 10 Countries
- Other Middle East
- Other North Africa
- Other South America
- Other Sub-Saharan Africa
- West African Community = (Ecowas (Minus Nigeria)
- Country
  - Argentina
  - Australia
  - Bangladesh
  - Brazil
  - Canada
  - China
  - Egypt, Arab Rep.

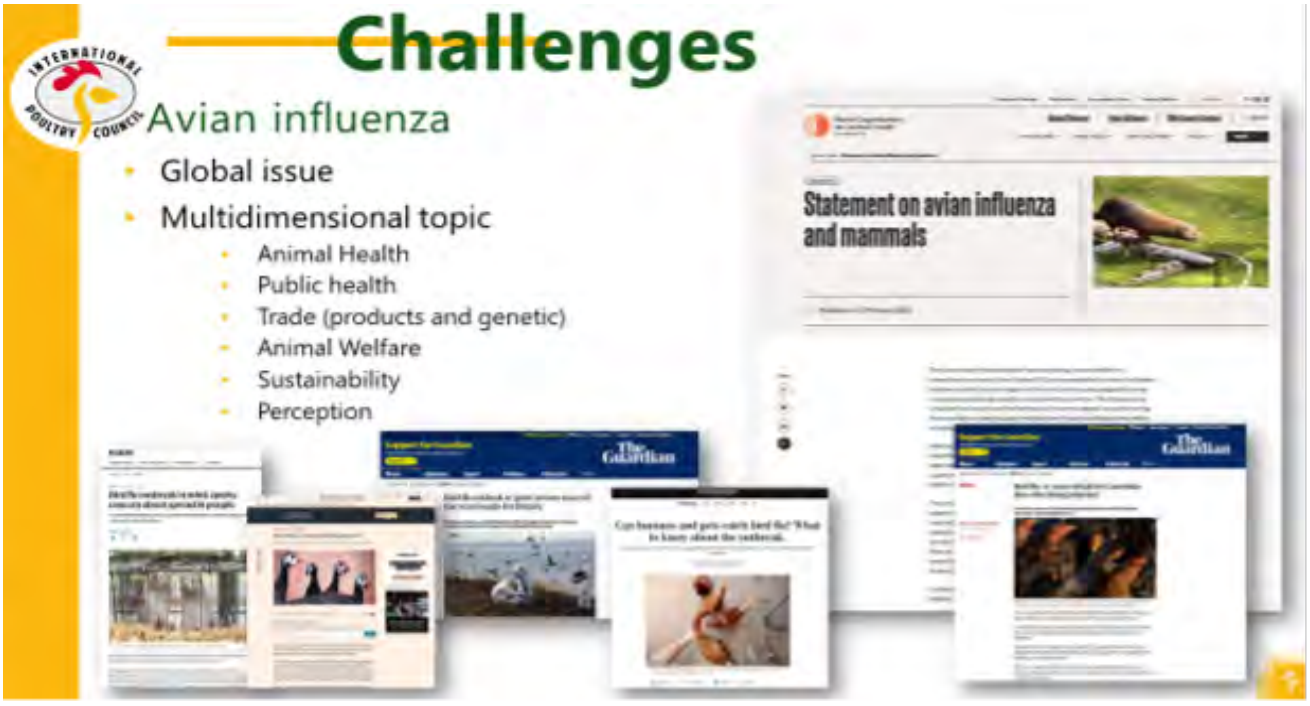
Select Protein:
- Beef and Veal
- Pork
- Poultry

Poultry Exports and Imports Projections (thousand tonnes)



Ratio between Projected Poultry Exports and Imports (thousand tonnes)


GLOBAL CONSUMPTION & PRODUCTION PROJECTIONS (USDA)
Select Publication Year:
2022
Select Market:
Bloc
Central America & Caribbean
European Union
Other Asia & Oceania
Other Europe
Other Former Soviet Union, 15 Countries
Other Middle East
Other North Africa
Other South America
Other Sub-Saharan Africa
West African Community = Ecowas (Minus-Nigeria)
Country
Argentina
Australia
Bangladesh
Brazil
Canada
China
Egypt, Arab Rep.
Select Protein:
Beef and Veal
Pork
Poultry
Poultry Production and Total Consumption Projections (thousand tonnes)
Production
Total consumption
120.000
110.000
100.000
90.000
2018
2020
2022
2024
2026
2028
2030
Ratio between Projected Poultry Total Consumption and Production (thousand tonnes)
0.960
0.955
0.950


Challenges
Avian influenza
Global issue
Multidimensional topic
Animal Health
Public health
Trade (products and genetic)
Animal Welfare
Sustainability
Perception
Statement on avian influenza and mammals
The Guardian

Amr and amu

Amr and amu - role of ipc

IPC has a robust stewardship program in place on AMR & AMU.

2017 IPC releases a "Position Statement on Antimicrobial Use and Antimicrobial Stewardship Principles" document.

2019 IPC releases a "Best Practice Guidance to reduce the need for antibiotics in poultry production", produced in collaboration with WOAH and supported by FAO

2021 IPC is a founding member of a consortium with Cargill, AusVet and Heifer International, appointed by USAID to run the 5-years project TRANSFORM

TRANSFORM, Tansformational Strategies for Farm Output Risk Mitigation, is the first 100% private lead project ever supported by USAID as part of its global health security agenda. Its goals are to increase global health security & access to safe, affordable animal-sourced nutrition through three integrated components:

DATA: Animal health data collection, analysis and application

INDUSTRY: Animal industry commitment to antimicrobial standards

ON-FARM: On-farm practices that support animal health and economic sustainability

2023 IPC and FAO collaborate to produce an FAO manual on AMR reduction

Role of poultry in global sustainable food security

- Perception vs reality
- Anti-livestock movements
- Need to produce more with less
- Sustainable and responsible food chain
- Animal Welfare
- Changing global
- Keep supply chain

WHAT'S NEXT?

- The global poultry meat sector is in good shape
- AI and AMR are two key topics to address
- The global geopolitical scenario could play a major role
- The poultry industry must take the lead at precompetitive level on high-priority topics
- IPC is actively promoting, leading, and facilitating coordinated actions to enhance the sector

INVITED

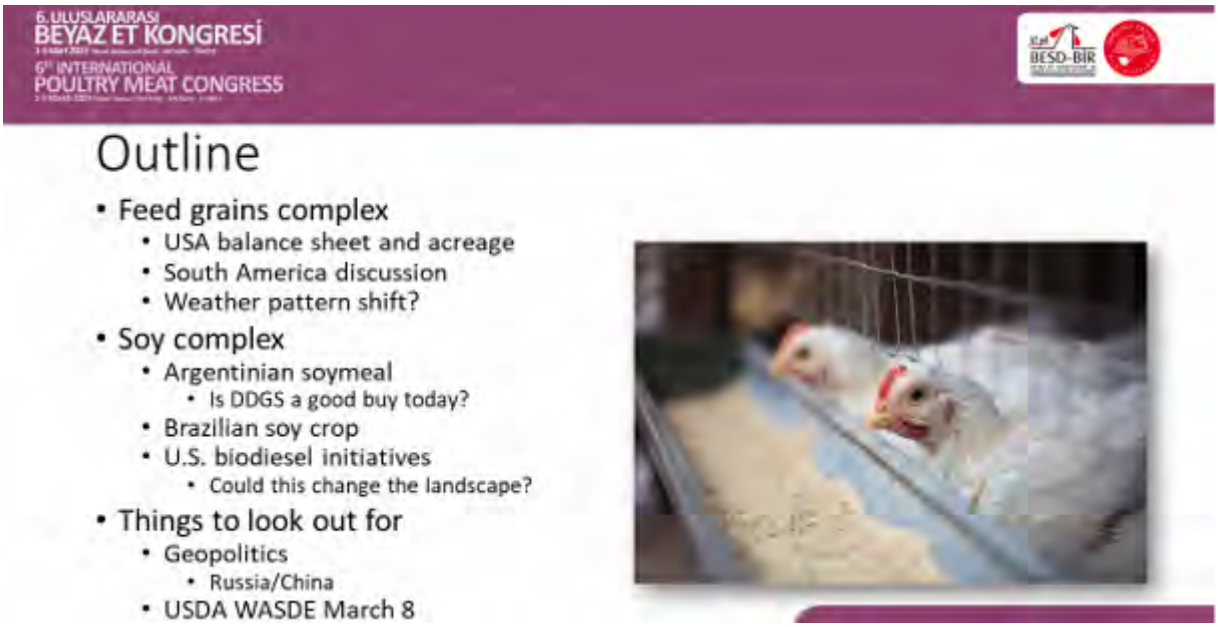
AND

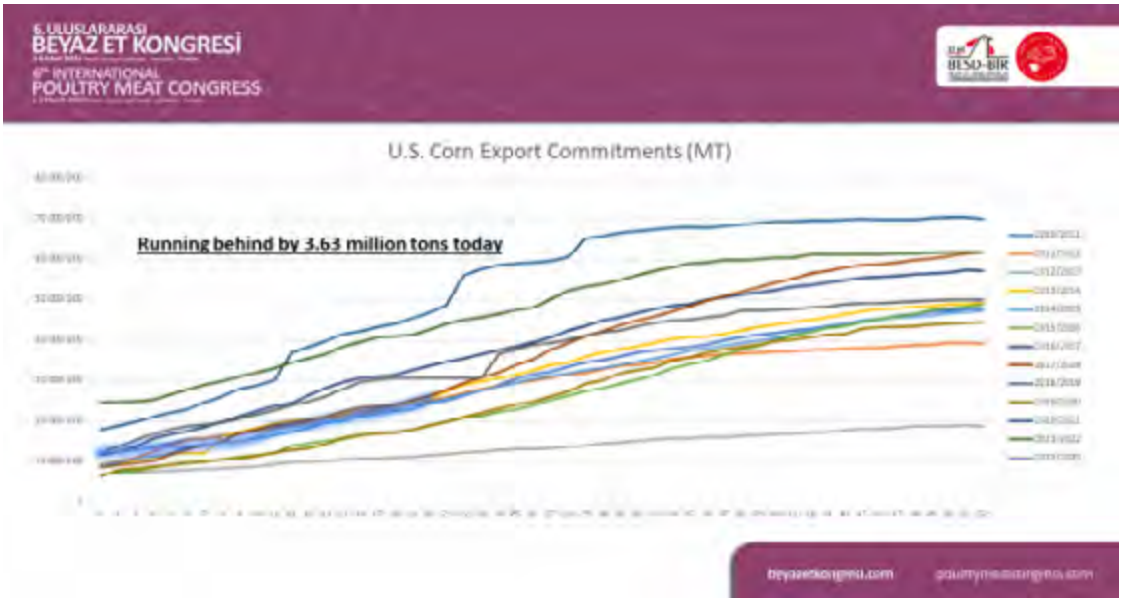
ORAL PRESENTATIONS

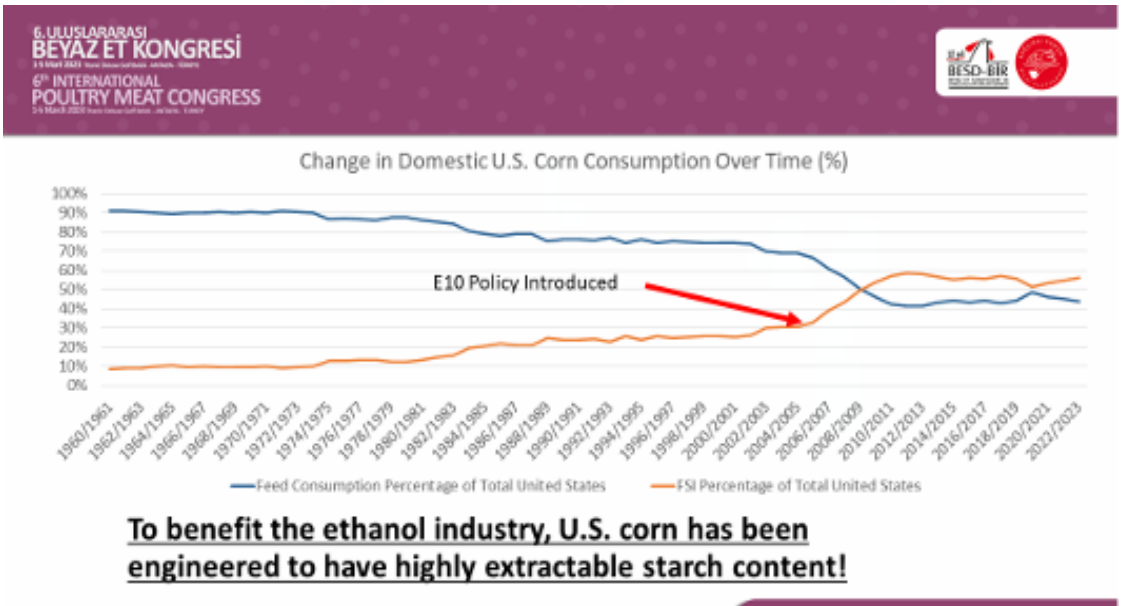
IS[01] Trade, Challenges for Grains and Protein Sources, and Future Expectations

Reece H. Cannady

US Grain Council, Washington, DC 20001,USA




6. ULUSLARARASI
BEYAZ ET KONGRESİ
6th INTERNATIONAL
POULTRY MEAT CONGRESS
U.S. Corn Export Commitments (MT)
Running behind by 3.63 million tons today


6. ULUSLARARASI
BEYAZ ET KONGRESİ
6th INTERNATIONAL
POULTRY MEAT CONGRESS
Change in Domestic U.S. Corn Consumption Over Time (%)
E10 Policy Introduced
Feed Consumption Percentage of Total United States
FSI Percentage of Total United States
To benefit the ethanol industry, U.S. corn has been engineered to have highly extractable starch content!


6. ULUSLARARASI
BEYAZ ET KONGRESİ
6th INTERNATIONAL
POULTRY MEAT CONGRESS
U.S. Weekly Ethanol Production (Average L/day)

6. ULUSLARARASI BEYAZ ET KONGRESİ
6th INTERNATIONAL POULTRY MEAT CONGRESS

S&P Platts

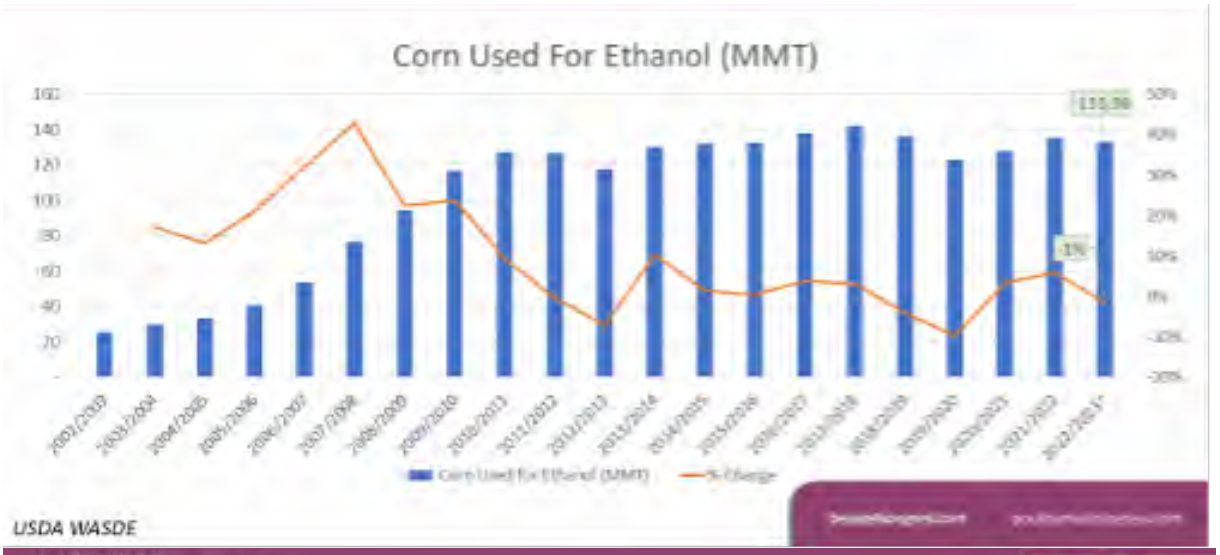
6. ULUSLARARASI BEYAZ ET KONGRESİ
6th INTERNATIONAL POULTRY MEAT CONGRESS

Impacts on Projected Ethanol Profits

- Strong oil sector
  - Chinese reopening looks to be happening post COVID wave
  - Increasingly doveish FED
- Natural gas price expectations cut 30%
  - Warm winter leads to higher than usual stocks for this time of year
  - Used to dry DDGS at ethanol plant
- Corn inverses getting larger

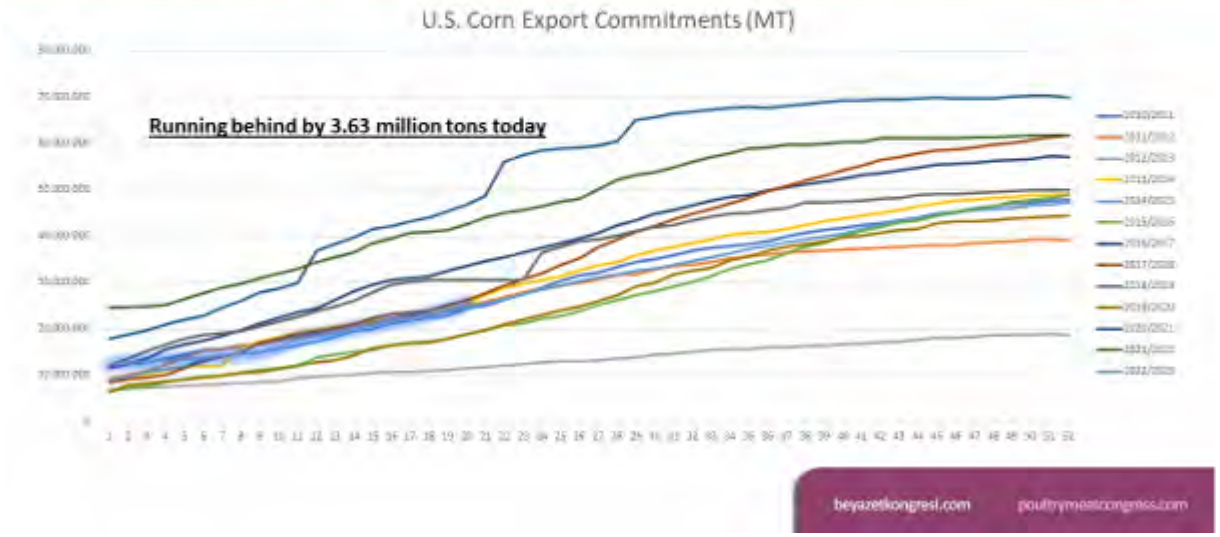
EIA lifts 2023 oil-price forecasts, but cuts natural-gas price outlook

Published: Feb. 7, 2023 at 12:36 p.m. ET



6. ULUSLARARASI
BEYAZ ET KONGRESİ
6th INTERNATIONAL
POULTRY MEAT CONGRESS

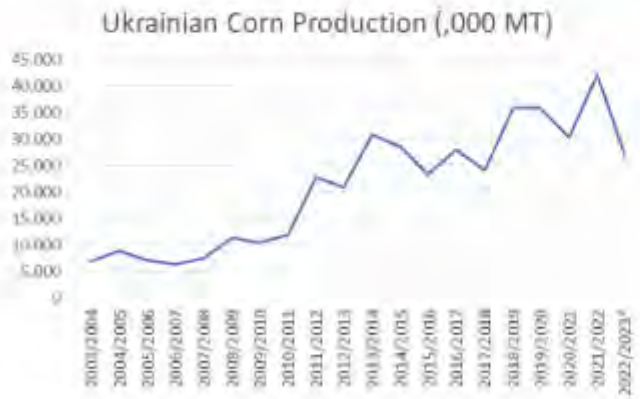
6. ULUSLARARASI
BEYAZ ET KONGRESİ
6th INTERNATIONAL
POULTRY MEAT CONGRESS



- Tensions growing, relationship souring...

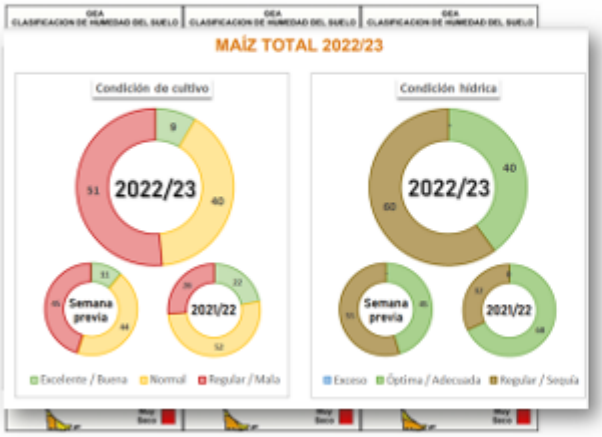
2022/23 Proj.		Beginning Stocks	Production	Imports	Domestic Feed	Domestic Total 2/	Exports	Ending Stocks
World 3/	Jan	305.95	1,155.93	175.45	732.95	1,165.47	178.17	296.42
	Feb	306.28	1,151.36	177.00	729.14	1,162.37	181.07	295.28
World Less China	Jan	96.82	878.73	157.45	516.95	868.47	178.15	89.10
	Feb	97.15	874.16	159.00	513.14	865.37	181.05	87.96
United States	Jan	34.98	348.75	1.27	133.99	304.56	48.90	31.54
	Feb	34.98	348.75	1.27	133.99	303.93	48.90	32.17
Total Foreign	Jan	270.98	807.18	174.18	598.96	860.91	129.28	264.88
	Feb	271.31	802.61	175.73	595.15	858.45	132.18	263.10
Major Exporters 4/	Jan	13.38	234.70	1.36	96.60	120.40	112.20	16.84
	Feb	13.68	229.70	1.36	91.60	115.40	114.20	15.14
Argentina	Jan	1.49	52.00	0.01	10.00	14.00	38.00	1.49
	Feb	1.49	47.00	0.01	8.00	12.00	35.00	1.49
Brazil	Jan	3.95	125.00	1.30	64.50	76.00	47.00	7.25
	Feb	4.25	125.00	1.30	61.50	73.00	50.00	7.55
Russia	Jan	0.93	14.00	0.05	9.80	10.90	3.30	0.78
	Feb	0.93	14.00	0.05	9.80	10.90	3.30	0.78
South Africa	Jan	1.92	16.70	0.00	7.30	13.30	3.40	1.92
	Feb	1.92	16.70	0.00	7.30	13.30	3.40	1.92
Ukraine	Jan	5.09	27.00	0.00	5.00	6.20	20.50	5.39
	Feb	5.09	27.00	0.00	5.00	6.20	22.50	3.39
Major Importers 5/	Jan	22.18	119.67	94.80	159.20	213.80	3.39	19.47
	Feb	22.20	120.07	96.30	161.10	215.70	3.59	19.28
Egypt	Jan	1.56	7.44	9.20	13.90	16.40	0.01	1.79
	Feb	1.56	7.44	9.20	13.90	16.40	0.01	1.79
European Union 6/	Jan	9.94	54.20	21.50	56.50	76.10	2.20	7.34
	Feb	9.94	54.20	23.50	58.50	78.10	2.20	7.34
Japan	Jan	1.38	0.01	15.00	11.50	15.00	0.00	1.39
	Feb	1.39	0.01	15.00	11.50	15.00	0.00	1.40
Mexico	Jan	3.16	27.60	17.20	26.00	44.20	0.60	3.16
	Feb	3.16	27.60	17.20	26.00	44.20	0.60	3.16
Southeast Asia 7/	Jan	3.40	30.26	16.48	38.10	46.35	0.58	3.13
	Feb	3.40	30.66	15.96	38.00	46.25	0.78	2.93
South Korea	Jan	2.06	0.08	11.00	8.70	11.05	0.00	2.09
	Feb	2.06	0.08	11.00	8.70	11.05	0.00	2.09
Selected Other								
Canada	Jan	2.75	14.54	1.00	9.30	14.50	1.60	2.19
	Feb	2.75	14.54	1.00	9.00	14.20	1.60	2.49
China	Jan	209.14	277.20	18.00	216.00	297.00	0.02	207.32
	Feb	209.14	277.20	18.00	216.00	297.00	0.02	207.32

- World carryout – 295.28 MMT
  - Down 1.24 MMT
  - Trade – 294.71 MMT
- Argentinian crop – 47 MMT
  - Down 5 MMT
  - Trade – 48.5 MMT
    - Might be more like 43 MMT today!
  - Exports down 3 MMT
- Brazilian exports at 50 MMT
  - Up 3 MMT – could they overtake U.S. for largest exports this year? USDA thinks so...
- Ukraine exports up 2 MMT
  - Strong pace reflected here



USDA WASDE

- Looking ahead – many considerations for next year's (2023) crop
  - Fertilizer
    - Did they have it bought this past year?
  - Planted Area
    - Can people get into the fields?
  - Labor
    - If they're still in wartime, do they have enough labor?



- Argentine corn yields projected 4th-lowest in 15 years
  - Lowest in 5 years
- Soil moisture saw help two weeks ago, but dryness continued into last week
  - Soil moisture continues to go lower, while g/ex follows

Could the U.S. Pick Up Argy Demand?

- During the last major Argentina production issue (17-18 crop year when prod declined to 33 MMT from 42 MMT originally) there were several countries buying more US
- Brazil may not have much to offer between March and June
  - China soaking up some of that corn
- US could benefit from exports depending on the severity of production losses in Argentina

Net Sales from Dec 2017 to Jun 2018

	28-Dec	28-Jun	Change	LY Total
BANGLADESH	500	115,511	115,011	
EGYPT	0	964,503	964,503	41,000
ISRAEL	0	458,803	458,803	183,000
KOREA, REPUBLIC OF	1,158,087	5,228,407	4,070,320	1,475,000
MOROCCO	72,472	679,702	607,230	256,000
SAUDI ARABIA	209,502	1,170,003	960,501	696,000
TAIWAN	315,843	2,311,580	1,995,737	633,000
VIETNAM	0	1,753,576	1,753,576	69,000
EUROPEAN UNION - 27	511,614	2,190,773	1,679,159	687,000
GRAND TOTAL	26,671,037	57,252,579	30,581,542	

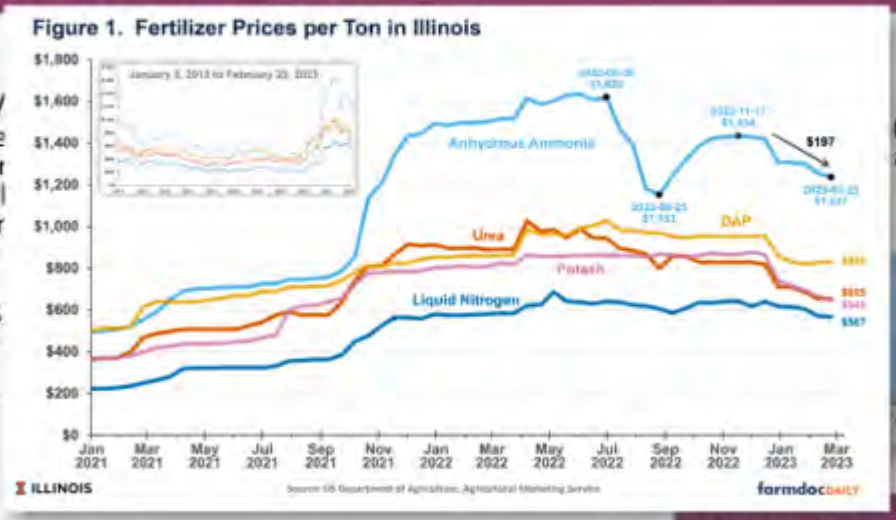
- Bulls and bears in deadlock
- Bulls
  - What if China comes back?
  - Brazil is planting safrinha late
    - El Nino could pose drought!
  - Black Sea risk premium
  - Argy drought risk
- Bears
  - China relationship souring
  - Brazil soil looks great
    - If El Nino comes, that means U.S. will have a huge crop!
  - Ukraine offers are thin outside of Med, anyway
  - Argy crop is getting better, why doesn't price break?
  - Huge acres coming—economics favor corn!
    - Fert prices breaking
  - What about feed wheat? Corn is pricing itself out of the market...

Corn finally prices back in vs. wheat

Global corn supplies price back in vs. wheat for first time in months! This was absolutely necessary for justification of these prices. Fundamentally, the market's fall last week was its work to be competitive.

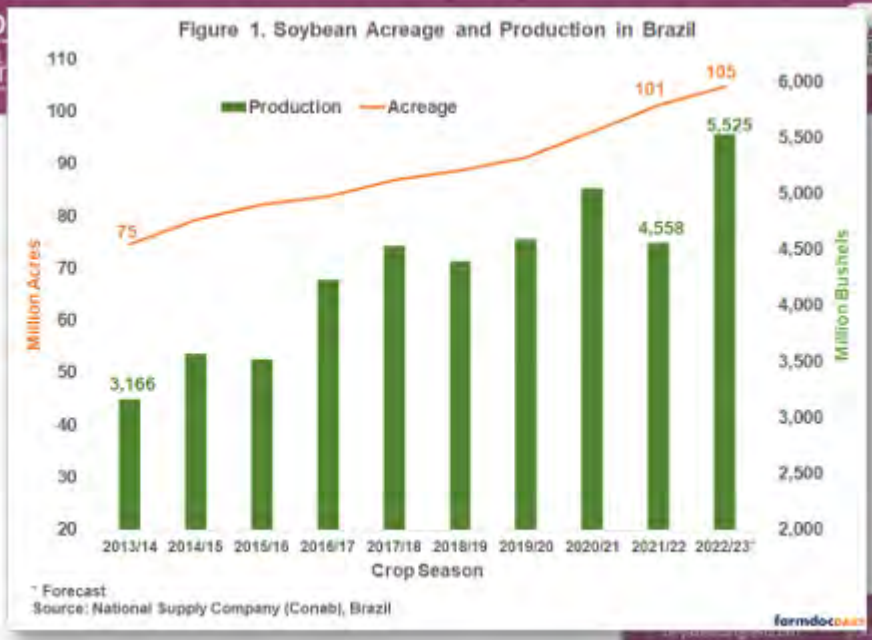
- Next y
  - The incr witl Ukr
  - U.S

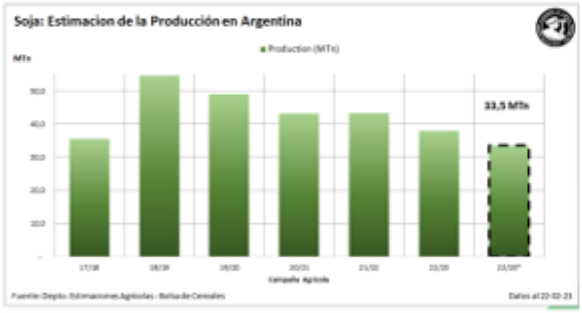
Figure 1. Fertilizer Prices per Ton in Illinois



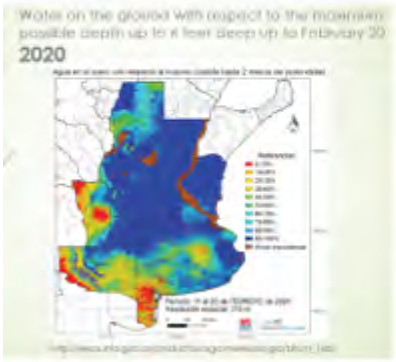
(87.5ma), and a Year Ago

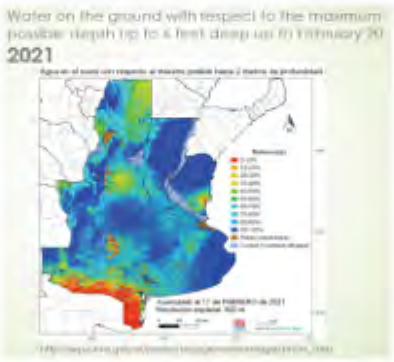
Figure 1. Soybean Acreage and Production in Brazil

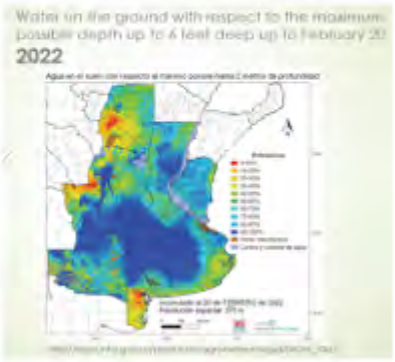




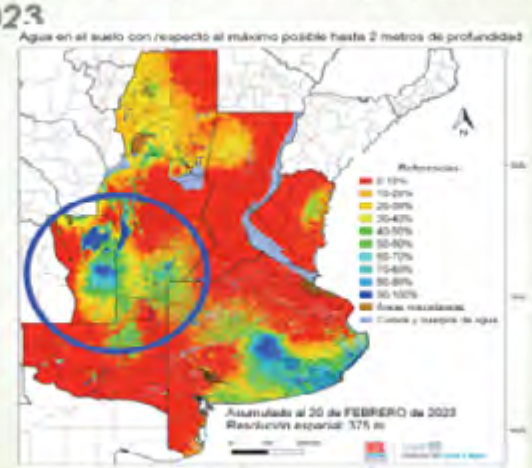
- Argentine soy production projected to be lowest since 08/09 crop year
  - Has driven global prices of meal up significantly
- Farmers will currently not offer their product, as they're unsure of what they're going to cut
  - Making soymeal offers also quite thin in the global marketplace



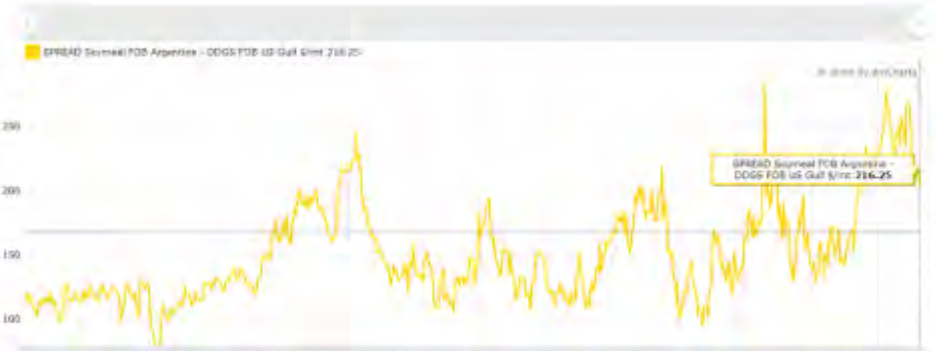




Water on the ground with respect to the maximum possible depth up to 6 feet deep up to February 20 2023



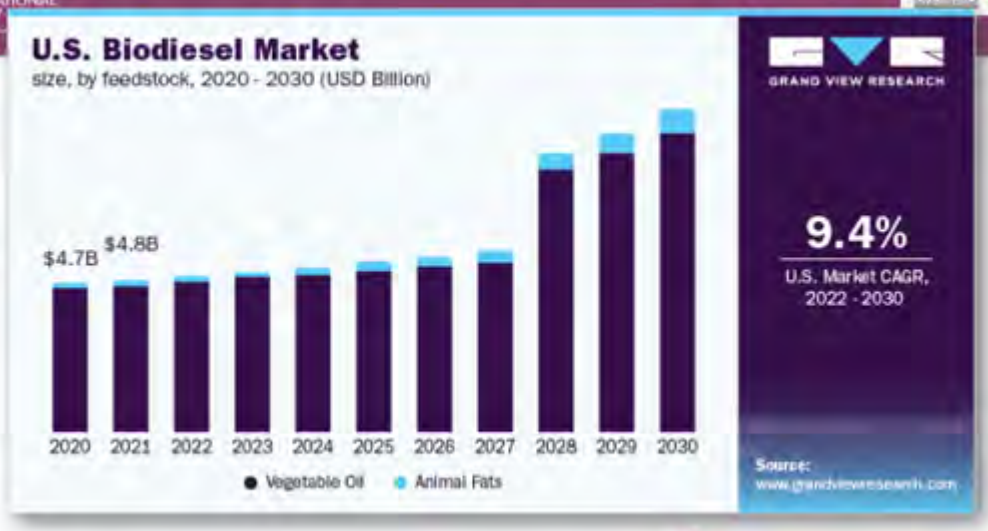
6. ULUSLARARASI BEYAZ ET KONGRESİ
6th INTERNATIONAL POULTRY MEAT CONGRESS

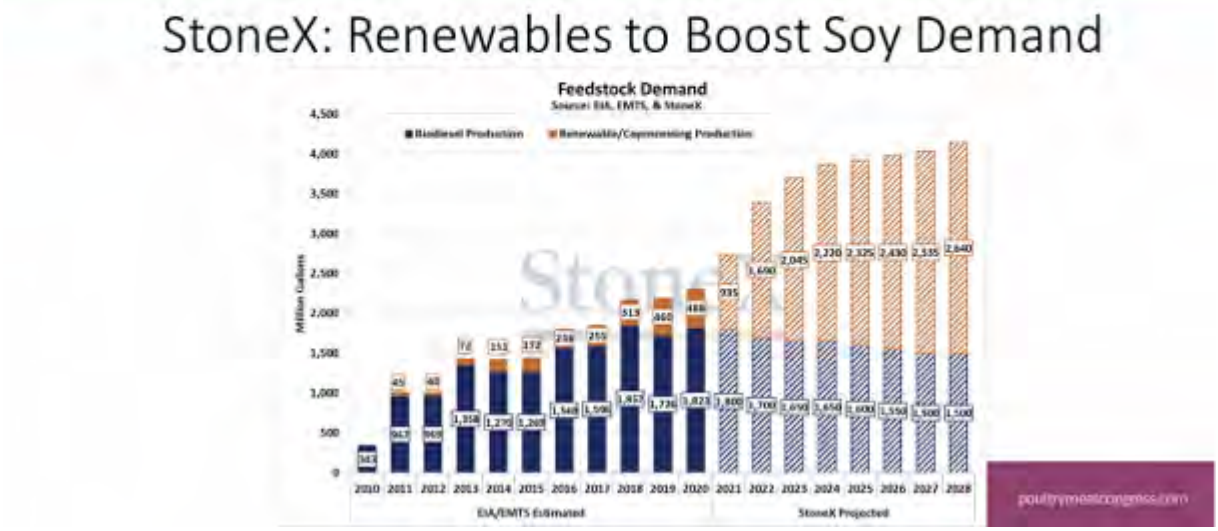


Rarely have we seen the spread between Argy soymeal and U.S. DDGS be so high

Great time to look at DDGS!

6. ULUSLARARASI BEYAZ ET KONGRESİ
6th INTERNATIONAL POULTRY MEAT CONGRESS

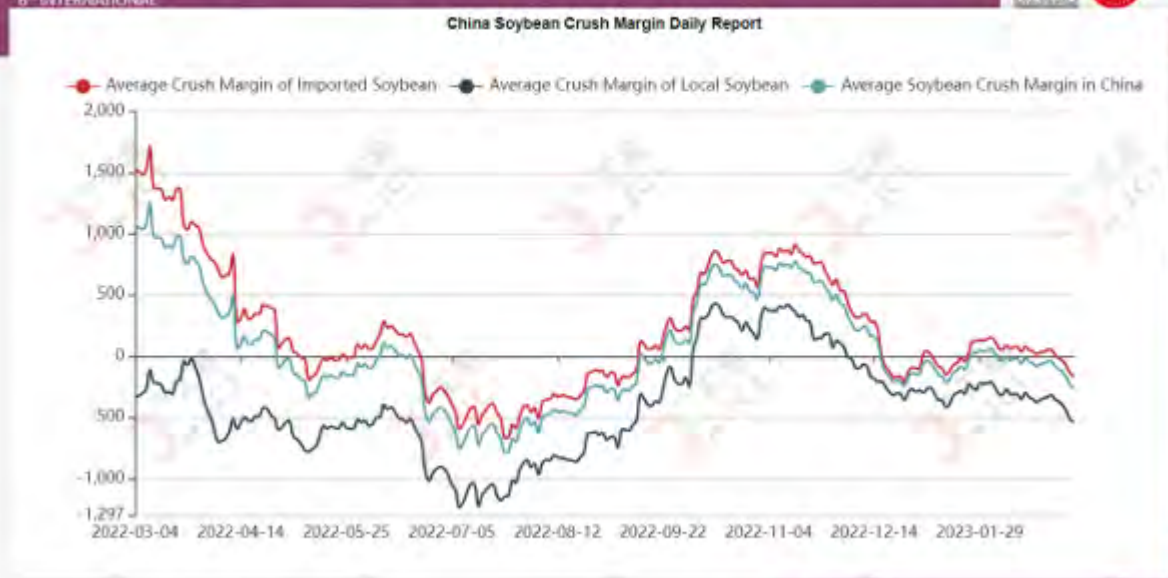




Incoming Soy Crush

New Plants / Expansions	Bushels / Day	Start Date	Port
Shell Rock, IA	110,000	Dec-22	GULF
ADM/Marathon, Spiritwood, ND	150,000	Fall 2023	PNW
Epitome Energy, Crookston, MN	120,000	Late 2023	PNW
Platinum, Alta, IA	110,000	Spring 2024	GULF
Bunge/Chevron, Cairo, IL	115,000	Late 2024	GULF
Bunge/Chevron, Destrehan, LA	115,000	Late 2024	GULF
Bartlett Grain, Montgomery Co,KS	110,000	Late 2024	GULF
CGB / MN SB Proc.,Casselton, ND	120,000	Late 2024	PNW
AGP, Sergant Bluff, IA	50,000	Late 2023	PNW
AGP, David City, NE	150,000	Mid 2025	PNW
Marquis Energy, Hennepin, IL	115,000	Late 2024	GULF
Norfolk Crush, Norfolk, NE	115,000	Late 2024	PNW
TOTAL	1,380,000	(37,500 MT/Day)	

Source: American Soybean Association



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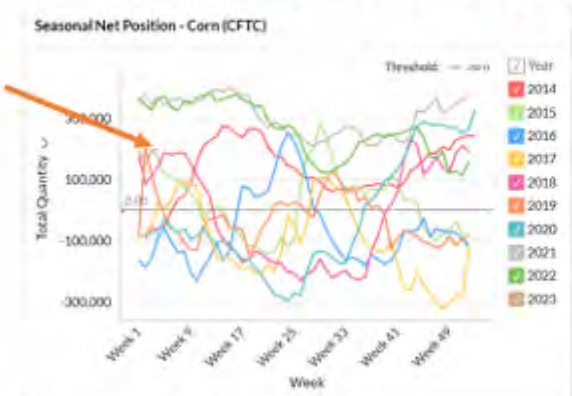
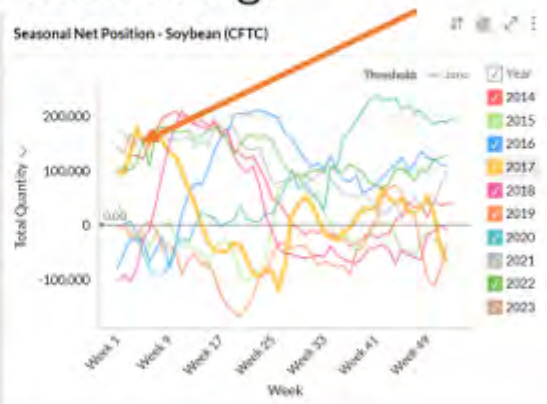
What are the funds doing?





Source: CFTC via Agricensus


6. ULUSLARARASI
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POULTRY MEAT CONGRESS
BESD-BİR
• Geopolitics
• Does the war in Ukraine end? Not likely to happen soon…
• China/U.S. tensions bubbling over
• Does China invade Taiwan?
• Leaked intel shows that this may not happen until 2027
• Is Brazil going to have the safrinha corn crop we are all expecting?
• Late planting!
• Will the U.S. acreage shift have a huge impact on production? Can we hit the expected yields USDA released a few days ago?
• Weather patterns – we are expected to shift to El Nino
• Drier Brazil, Australia, and South Africa
• Wetter Argentina, US
• Biodiesel mandates and what this means for U.S. soy crush
• Huge trend that cannot be ignored
• Does the U.S. become a meal seller?
beyazetkongresi.com
poultrymeatcongress.com

IS[02] Improving Welfare and Sustainability of Poultry Meat Production

Anne-Marie Neeteson-van Nieuwenhoven[1], Santiago Avendaño[1], John Ralph[2], Tim Burnside[1]

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Abstract

Poultry meat is lean, healthy and affordable with a low environmental impact. Animal breeding has contributed to these attributes and to the improvement of welfare at the same time. Breeding is at the start of the food chain and is a long-term exercise. Breeding outcomes are permanent and cumulative and disseminated widely through the supply chain. Breeding for welfare and sustainability requires simultaneously improving and optimising environmental impact, productivity, robustness and welfare. Over recent decades, meat poultry breeding programmes have implemented balanced approach by broadening breeding goals and managing trait antagonisms. Continued investments in all parts of the breeding programme, together with feedback from all stakeholders across the production chain, ensure the direction of progress is in line with the requirements and needs of the customer base and society as a whole.

Introduction

At Aviagen, chicken and turkey welfare is a cornerstone of sustainability. Animal welfare provides a crucial contribution to sustainable meat production: healthy animals are more resistant to disease, their liveability is stronger, and they perform better. These are also positive for the environment and economic sustainability of farmers, meaning welfare and sustainability is essentially good business. This is possible because antagonistic biological outcomes like those between production and welfare and health can be and have been improved at the same time leading to a choice of crossbreeds with improvements in a broad range of characteristics.

Breeder commitment to improving sustainability

Within Aviagen a set of 5 commitments to breeding for welfare and sustainability have been established alongisde supplying global food demands, have been established (Aviagen, 2018; Aviagen Turkeys, 2017). The 1st Commitment is around health and food security, securing a safe and secure supply of healthy birds to help feed a growing global population. The 2nd commitment focuses on genetic diversity through maintaining broad gene pools, the major asset of a breeding company. The 3rd commitment relates to implementation of a balanced breeding programme ensuring harmonised progress across all traits in the breeding goal. The 4th Commitment is around the importance of management and stockmanship of the improved populations which allows the expression of genetic potential across all production systems around the world. The 5th Commitment is covering transparency, communication and engagement. These commitments are in line with the Code of Good Practice for Sustainable and Responsible Animal Breeding, Code-EFABAR (EFFAB, 2020; Besbes & Neeteson-van Nieuwenhoven, 2006), and aligned to the global meat sector sustainability commitments (Aviagen, 2021). The International Poultry Council (IPC), the global meat poultry association, has established where the meat poultry sector can make a difference for sustainability, in terms of the three pillars of environment, economy and society, and of the United Nations Sustainable Development Goals, SDGs. These are in particular SDGs 2 No Hunger, 3 Good Health and Well Being, 4 Quality Education, 9 Industry, Innovation and Infrastructure from and with good health and welfare, and 13 the Climate: where poultry impacts positively on our global footprint. This alignment with the UN SDGs was confirmed in a joint Food and Agriculture Organisation (FAO) - IPC declaration, signed in 2019 in São Paulo, Brazil (IPC, 2019).

The Aviagen breeding programmes

Since the 1970's Aviagen has demonstrated an ever-increasing focus on animal welfare and sustainability traits within its broiler and turkey breeding programme. Aviagen keeps large gene pools consisting of a wide range of pedigree chicken and turkey strains with a very wide range of biological features and attributes. These populations give rise to the great-grandparent and grandparent generations which in turn originate the parent stock and then the commercial broiler or turkey crossbreds. It takes around four years from pedigree to the broiler/turkey generation – resulting in performance and welfare-

improved birds, with at the same time better health and environmental impact. These improvements are continuous and cumulative over time. It is crucial that at all times trends and requirements from customers and society are considered when setting the breeding direction. In addition, a key component of sustainable breeding is continuous investment in research and development to follow the latest scientific insights and technological advancements.

Selection of antagonistic traits: It is not taken for granted that all traits are improved at the same time, as when only production traits are improved then health, welfare, robustness and reproduction can be impacted negatively. This antagonism of traits can be overcome as long as the genetic correlation (GC) between the traits is not extreme. Figure 1 shows the GC between a number of health and welfare traits with body weight and yield in broilers (Avendaño *et al*. (2017)).

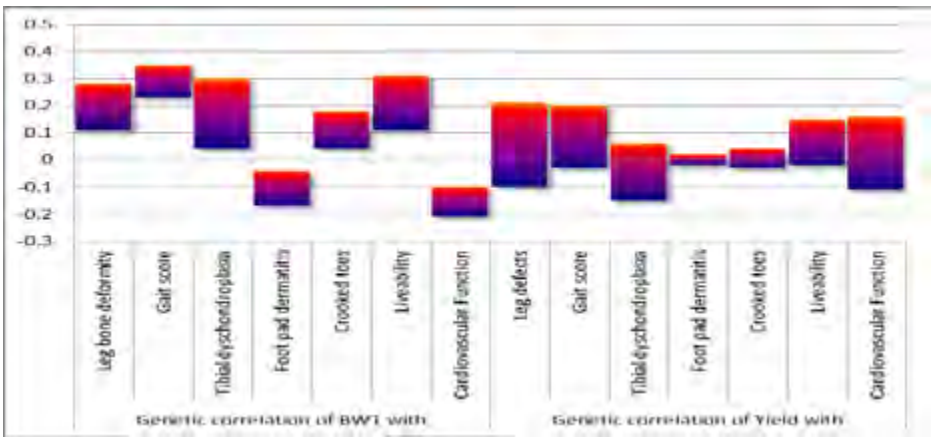

Figure 1. Ranges of genetic correlations between live weight (BWT) and breast yield (%) with leg bone deformities (%), gait score, Tibial Dyschondroplasia (%), Footpad Dermatitis (%), crooked toes (%), liveability (%) and oxygen saturation levels in blood (%; cardiovascular function). [From: Avendaño *et al*., 2017]

The antagonism between traits can be managed if accurate phenotypes of each trait is recorded and both traits involved in the antagonism are included in the breeding goal. The more unfavourable the relation between traits, the more efforts (being more precise, measuring more animals etc.) must be made to reach improvements (Hocking, 2014).

To illustrate this, Avendaño *et al*. (2017) showed two antagonistic traits in broiler breeding: feed conversion rate (FCR) and hatchability (Figure 2). In this example, the GC between hatchability and FCR is 0.27, a typical antagonism between meat bird and breeder performance traits. The trend line indicates that moving to the left the birds have better FCR but worse hatchability while to the right the birds have improved hatchability but poorer FCR. In this example FCR would deteriorate by 0.012 (i.e., 12 g of feed per kg of live weight) per 1% improvement of hatchability. The antagonism is overcome by including both traits in the breeding goal and selecting birds that have both good FCR and hatchability. These birds are in the bottom-right box. Following this approach, all traits in the breeding goal can be improved simultaneously.

In broiler and turkey breeding programmes with tens of traits each, these antagonistic as well as positive relationships between the different traits are considered to ensure a holistic improvement of all traits over time. At the same time differentiation and specialisation between lines for different roles in the crossbreed product is maintained allowing a different breeds to be offered to the market (Ralph, 2017a).

Improvements in welfare :Improvements in welfare are realised by managing trade-offs between performance traits (e.g., body weight, breast yield%, FCR) and welfare related traits (e.g., leg health, contact dermatitis). Kapell *et al.* (2012a, b) confirmed that leg health (e.g., valgus / varus) has effectively been improved for decades, and showed how contact dermatitis (e.g., footpad health) can be improved simultaneously in high and low hygiene environments by managing the information from pedigree birds and their siblings in different environments in an intelligent breeding programme design. This illustrated that "continued genetic improvement for production efficiency and minimisation of demand on resources is feasible without sacrificing animal health and welfare in a multi-trait selection set up including both fitness and production traits" (Hill, 2016).

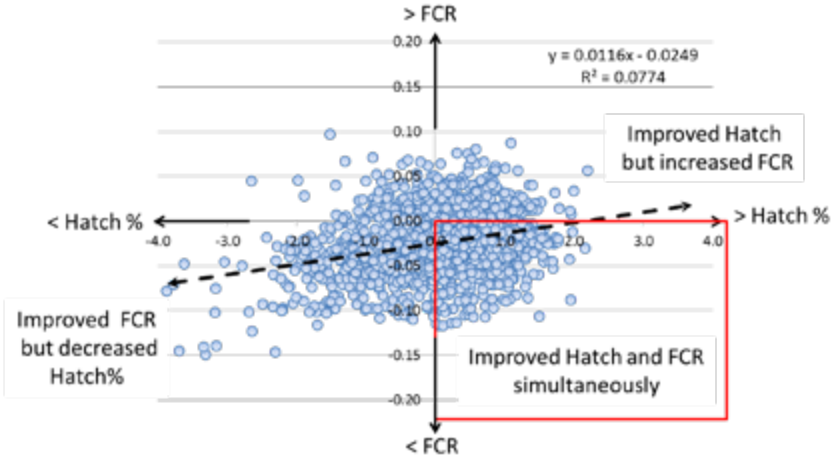

Figure 2. Estimated Breeding Values (EBVs) for 1385 pedigree birds for FCR (Feed Conversion Rate; vertical axis) and hatchablity% (horizontal axis) are shown as deviations from the population mean. [From: Avendaño *et al*., 2017]

The simultaneous welfare and production improvements between 1996 and 2022 of the pedigree lines comprising the Ross 308® are illustrated in Figure 3. Although the relationships between body weight and the health and welfare traits are unfavourable within each year, there is a clear favourable direction for each trait because of simultaneous selection, that is, as bodyweight increases, cardiovascular function, leg strength and liveability increase while cooked toes decreases.

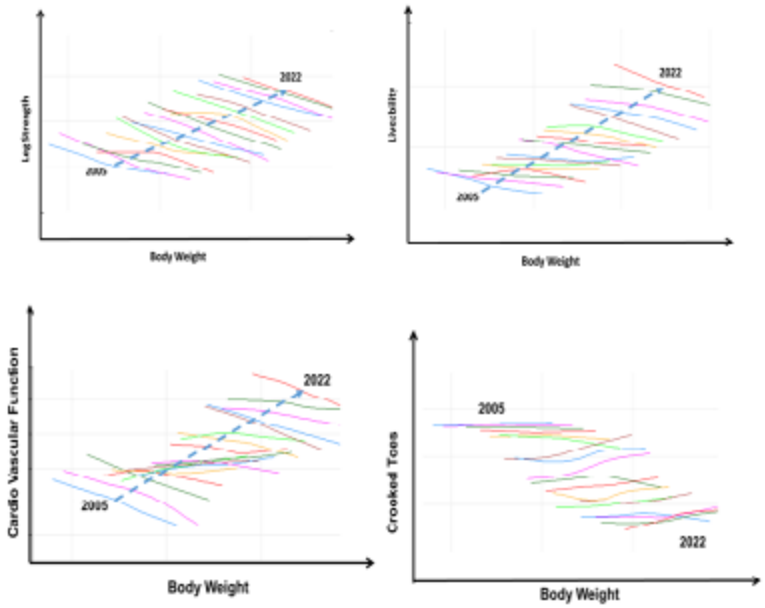

Figure 3. Long term relationships between live body weight and leg strength (%), liveability (%), oxygen saturation in the blood and crooked toes (%). Each coloured line represents the relationship between breeding values for each trait within a year. The broken arrow represents the joint direction of the average breeding value for each trait involved in the trade-off

Similar means to the improvement of health and welfare and achieved advances in turkeys have been explained and illustrated in walking ability, footpad health, valgus / varus and tibial dyschondroplasia (Maine *et al.* 2006, 2007; Swalander, 2012; Kapell, 2016; Kapell *et al,* 2017; Ralph, 2017b).

Public information from the Canadian Food Inspection Agency (CFIA, 2023; Figure 4) show that the results in the breeding programme are also reflected at the industry level – ascites, reflective of heart and lung health, and leg health have demonstrably improved between the 1990s and 2022 in broilers and turkeys.

Improvement of robustness :How robust meat chickens and turkeys are globally is very relevant as the birds are farmed across the world in a wide range of production regimes. The diversity of environments can be characterized by varying management practices, feed quality (form and density) and gut and immune challenges. In these environments substantial genotype × environment (G×E) interactions will exist (Hill *et al*., 2016).

Whereas pedigree populations are kept at high standards of management, health and bio-security, it is crucial to include elements of differential production to the pedigree programme to minimise the GxE interaction and ensure the expression of the genetic potential in commercial environments. One way to do this is to combine records from high hygiene pedigree environments with those taken in low biosecurity, low input environments to represent the wide range of commercial production environments in selection decisions, e.g. via commercial sibling testing (CST; Kapell *et al.*, 2012a). Using CST

improves robustness because siblings will express a range of traits related to robustness and environmental adaptability like gut health, digestive, and immune function along with liveability, growth, and uniformity. The result will be improved productivity in both environments, increased animal adaptability to the wider range of management circumstances they may encounter in the field, and more robust populations with higher liveability and uniformity (Ralph 2016).

Improvements in environmental footprint : Population growth and prosperity is fuelling demand for global meat production and increasing the importance of its environmental impact. Poultry production is in a favourable position in terms of environmental impact with 3.7-6 kg CO_2 per kg edible carcass which is much lower than the corresponding ranges for other meats (20-60 for ruminants and 7-20 for pork) (Poore and Nemecek, 2018; Ritchie and Roser, 2020). Poultry is expected to have a pre-eminent role in satisfying the increasing global demand for meat products while at the same time reducing the environmental impact of meat production globally. Recent predictions suggest that Green House Gas emissions of the meat sector are projected to rise by 9% by 2031 which is less than the 15% increase in total meat production due to the shift towards poultry production (OECD-FAO, 2022).

With a Life Cycle Assessment (LCA) tool developed by Cranfield and Newcastle University (Poultry LCA Model Version 1.0) (Williams *et al,*, 2006; Leinonen et al., 2012) Aviagen recalculates the environmental impact of the broiler crossbreeds in the portfolio on a regular basis (e.g. Avendaño *et al.,* 2017). The 'cradle to farm gate' LCA approach accounts for all inputs and outputs of a production system as described by Leinonen *et al.* (2012). Ross 308® and 708® are globally established commercial broiler crossbreeds; Rowan Range® broiler types are slower-growing crossbreeds (eu.aviagen.com/brands/rowan-range). The Rowan Range genotypes fall under the requirements of accreditation schemes like Global Animal Partnership (GAP)'s Better Chicken in North America (GAP, 2023), 'Kip van Morgen' (ACM, 2015) and 'Beter Leven' (Dierenbescherming, 2023) in the Netherlands, 'RSPCA Assured' (Royal Society for the Prevention of Cruelty to Animals (RSPCA), 2023) and Red Tractor Enhanced Welfare (Red Tractor, 2023) in the UK, and 'Für mehr Tierschutz' (Deutscher Tierschutzbund, 2023) in Germany.

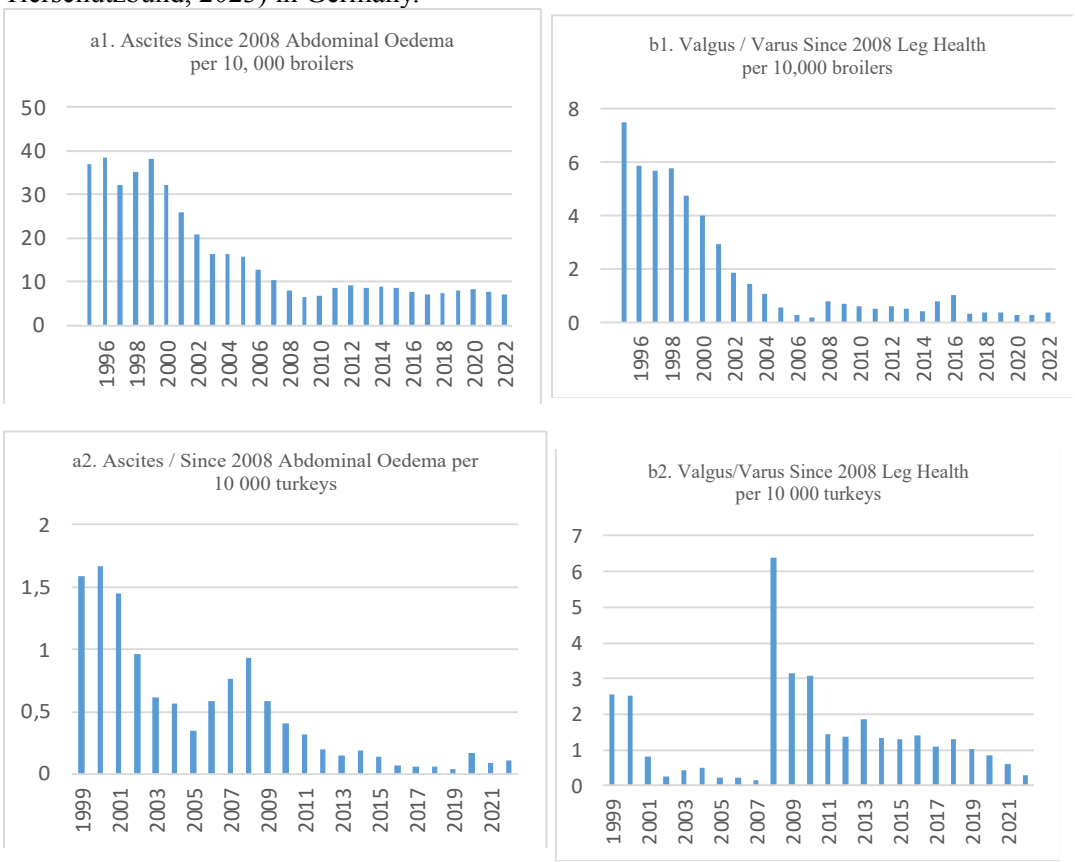

Figure 4. *Ascites (as from 2008 abdominal oedema) (a1 and a2) and leg health (until 2007 valgus/varus) (b1 and b2) related condemnation rates in broilers/turkeys per 10,000 (1. Chickens 1995-2022; 2. Turkeys 1999-2022). Canadian Food Inspection Agency (CFIA), 2023)*

More biologically efficient crossbreeds have the lowest environmental impact in terms of pollutant emissions and other outputs in LCA (Figure 5). As average daily gain and breast yields increase, global warming potential (GWP) decreases

linearly - the opposite trend is seen for feed conversion rate (FCR). These results are consistent with the findings by Leinonen *et al*. (2012): a free range and an organic production system had a predicted higher GWP of 16% and 28%, respectively over a standard production system. These findings are in line with Herrero *et al*. (2013) which conclude that feed efficiency is a key driver of productivity, resource use and greenhouse gas emissions.

The overall conclusion is that the environmental impact of crossbreeds with lower biological efficiency on resource utilisation and environmental burdens is 30-40% higher compared to conventional genotypes. FCR differences were also the main driver for environmental impact differences in LCA research in turkeys (Kremer *et al.,* 2014, Ralph 2020) and in a study comparing male and female turkeys grown in two ventilation systems (Leinonen et al., 2014a, b; 2016). On the other hand, more broadly, the suitability of a crossbreed to a production system or market segment will depend not only on biological performance but also on consumer preference, product price and other product attributes including the perceived importance between performance and welfare (Avendaño *et al.*, 2017).

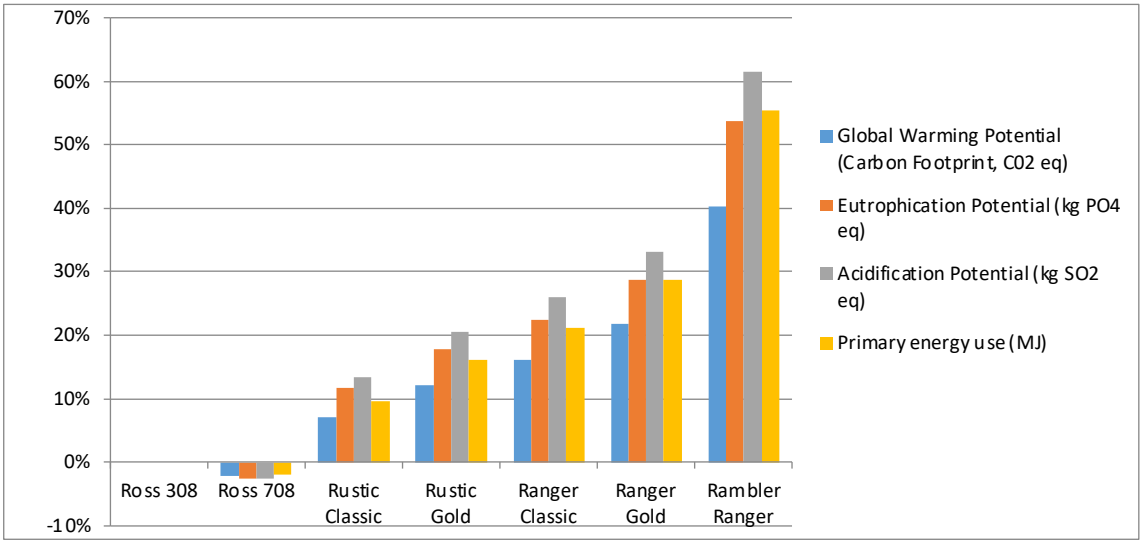

Figure 5. Relative global warming potential (GWP), acidification potential (AP), eutrophication potential (EP) and primary energy use (PEU) levels for seven Aviagen crossbreeds to 2.5 kg bodyweight (Ross 308 is the base for comparison at 1.0)

Discussion

Poultry breeding takes place at the start of the food production chain, and its improvements are permanent (in the genes of the animals), cumulative (the changes from one generation is added to the improvements of previous generations) and are disseminated to the wider industry

Poultry meat is an increasing part of animal protein in human diets and its role feeding humans continues to gain importance. A critical look at poultry production is, therefore, understandable and for the poultry sector to embrace and work diligently on continuous improvements. Data to show the improvements in productivity, health and welfare, production and environmental impact across a broad spectrum of crossbreeds will illustrate the achievements that have been made. Ideally the improvements will be reflected in the opinion about poultry production in the wider society.

An example of criticism about breeding broilers can be found in the EFSA (2010a, b) scientific opinions, which stated amongst others that "*The major welfare concerns...have a genetic basis and.. may interact with management factors to lead to poor welfare include skeletal disorders, contact dermatitis, ascites and sudden death syndrome. Most of these are linked with fast growth rates."* Indeed, with unbalanced breeding this could be the case. Later, the Farm Animal Welfare Council (FAWC) Breeding report (FAWC, 2012), and the European Commission report by Hiemstra and Ten Napel of 2013 did confirm that breeding goals in meat poultry have for decades included traits on skeletal, leg and foot health, heart function, liveability. FAWC (2012) stated that with regard to the concerns 'summarised in 2004' (FAWC, 2004) they were 'encouraged that many breeding goals now include aspects of animal welfare', and that 'as long ago as the 1970s some breeding companies recognised the benefits of including welfare (e.g. leg health) in tandem with production traits in their selection programmes.' For Aviagen, this highlights the importance of our commitment to transparency, communication and engagement.

In addition, information about the ways to deal with antagonism, and improvements achieved have come available from peer-reviewed literature (e.g. Kapell et al., 2012a, b; 2017, Kapell 2016)), and are demonstrated in public information like

the Canadian Food Inspection Agency data (CFIA, 2023), articles in poultry magazines, and in more public friendly form from livestock sector actors like the European Livestock Voice (ELV, 2022), the Animal Agricultural Alliance (AAA, 2023), European Forum of Farm Animal Breeders (EFFAB, 2022, 2023), and the company and association websites (e.g. Aviagen 2021, 2023; Aviagen Turkeys, 2023), social media messages, webinars and continuous dialogue with the wider stakeholders including governmental bodies.

Next to IPC with their commitment to the UN sustainability pillars and SDGs, globally and at more regional levels, the poultry sector have developed welfare and sustainability initiatives. In addition to the voluntary auditable standards like National Chicken Council (USA; NCC, 2020, 2022), National Turkey Federation (USA; NTF, 2023), Red Tractor (UK; Red Tractor, 2023), Qualität und Sicherheit (Germany; QS, 2023), the US poultry sector have developed a Sustainability Framework (US-RSPE, 2023), the European meat poultry sector the Sustainability Charter (avec, 2023), and the International Poultry Welfare Alliance (IPWA) Key Welfare Indicators (KWIs) (IPWA, 2023). These are all instruments aimed to show how the poultry sector works with sustainability and welfare.

Aviagen has a long standing commitment to contributing to food security, whilst improving animal welfare as well as better health, robustness, and environmental sustainability and product base for options from affordable protein to the diversity of production systems for both conventional and slower-growing birds. At least one-third of the selection focus is on health and welfare traits. Ongoing innovation is at the heart of the company – with innovative technology, current traits will be improved and new areas explored and where feasible implemented. Examples are in gut health, behaviour, and computed tomography scanning to assess both meat yield and welfare in live birds.

In addition, from the 1st Commitment we continue to work for a safe and secure supply of healthy birds, free from diseases like Avian Influenza, Newcastle Disease, Leucosis, all Salmonellae and all Mycoplasmas, with breeding programmes on two continents and high-generation operations spread around the globe. The 2nd Commitment, the diversity of poultry lines as the basis we breed from, covers our responsibility to ensure there is poultry available for the future, and to provide a choice of cross-breeds. With the outcome of the 3rd Commitment, the balanced breeding programme, the bird populations change on an ongoing basis, and the way to manage them will change gradually over time, as will the conditions poultry farmers will encounter due to technological and societal changes. At the flock level a large part of the health, welfare and performance are influenced by management factors. The Greenwell project (De Jong and Te Beest, 2020; De Jong *et al.,* 2022) illustrates that with the fast and slow-growing breeds used in the Netherlands the farming system was the major factor impacting outcome-based bird welfare. Management was the key factor explaining the differences between the conventional system (fast-growing birds, standard conditions), Kip van Morgen (broilers with maximum 50-gram growth per day, farming conditions close to Better Chicken Commitment) and Beter Leven 1 star (maximum growth per day of 45 grams, additional management requirements like lower stocking density, environmental enrichment, early feeding of day old chicks). The 4th Commitment represents our investments in customer support like management materials, technical specialists, and training schools. Last but not least, the 5th Commitment, engagement, transparency and communication, supports our efforts to be transparent about our breeding work through publishing articles for peer-reviewed scientific journals, trade publications, workshops/schools, and our website. This includes actively engaging at various levels via customers and associations with the wider society. It is important we enter the dialogue, explain what we do and show how we are committed to breeding welfare and sustainability which is also good business.

Conclusion

Broiler and turkey breeding will continue to focus on improving overall welfare, health, productivity and environmental impact using broad balanced breeding programmes. The portfolio of commercial broiler and turkey crossbreeds will keep expanding following the requirements of the poultry industry and consumers ensuring the sustainability of poultry breeding.

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OP[01] Effects of Barrier Perch Access and Early Dietary Protein and Energy Dilution on Some Welfare Parameters, Tibiotarsus Measurements in Broilers

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Abstract

His experiment aimed to determine the effect of increasing mobility by placing barrier perches between feeder-drinker and limitation of early weight gain by dietary energy and protein dilution on some welfare parameters, tibiotarsus measurements and mobility level. A total of 504 male Ross 308 1-d-old broiler chickens were allocated to four treatments in a completely randomized design with 2 x 2 factorial arrangement of barrier perch-BP (absence-/presence+) and diluted diet-DD (absence-/presence+). Some welfare parameters (foot pad dermatitis, hock burn, gait score, breast blister), tibiotarsus measurements (bone mineral content, bone mineral density, fluctuating asymmetry and relative fluctuating asymmetry) and mobility level were recorded. Results showed that access to barrier perch and diluted diet have increased the mobility level of broiler chickens. It was determined that access to barrier perch has no significant effect on tibiotarsus and welfare parameters. It was found that broiler chickens had a better gait score ($P<0.05$) and lower foot pad dermatitis incidence ($P<0.01$) in treatments with a diluted diet. It was detected that the diluted diet has no significant effect on bone mineral density but it reduced the tibiotarsus bone mineral content ($P<0.05$) in broiler chickens. In conclusion, it can be said that the diluted diet provides positive effects in terms of leg health problems due to weight gain limitation in the early period, thus improving broiler chicken welfare.

Keywords: Broiler chickens, barrier perch, qualitative feed restriction, welfare, dual-energy X-ray absorptiometry

OP02-Relationship between Feed Restriction and Blood Leptin Levels Under Heat Stress in Broiler Chickens

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Abstract

The present study aimed to investigate relationships between feed restriction and serum leptin concentrations in broilers under heat stress. A total of 480 day-old broiler chicks were reared under standard management conditions up to 34 days. At 34 days, broilers were divided into 2 groups, first group was kept as control while broilers in the second group were exposed to heat stress (33±1°C) between 10:00-16:00 hours until 37 d. Chickens in each group were randomly divided into two groups; half of them were fed *ad libitum*, while feed was restricted for the other half during the heat stress. Feed intake and body weight gain decreased, while rectal temperature increased under heat stress. Under heat stress and feed restriction condition, serum leptin concentrations increased, while T_3 levels reduced. An increase in serum leptin levels and a decrease in serum T_3 levels resulted in a decrease in feed intake and helped to improve thermoregulation, consequently.

Keywords: Broiler chicken, heat stress, feed restriction, thermoregulation, leptin.

IS[03] Feed and Nutritional Strategies for Sustainable Chicken Meat Production

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Introduction

Sustainability is derived from the Latin word "sustinere" (Onions 1964) which has the meanings of protecting, supporting, upholding or enduring for a long time. Considering these meanings, sustainability; It can also be defined as the ability to maintain existence for a long time. Sustainability for people is the continuity of well-being in terms of environmental, economic and social structure; requires the responsibility of managing resources in accordance with this concept. It is of great importance that life cycle assessments (LCA) are carried out correctly in realizing sustainability on these three pillars. LCA is a methodology for conducting environmental impact assessments at all stages of the life cycle of a commercial product, process or service.

Area usage per kg protein production in poultry meat is 37 times lower than in extensive beef cattle and approximately 3 times lower than in intensive beef cattle. In terms of greenhouse gases (kg CO_2 equivalent) emissions in poultry meat production, it is 13 times lower than extensive beef cattle and 8 times lower than intensive beef cattle. Consequently, poultry meat production (in terms of kg protein production) has a lower environmental impact than that of beef cattle.

Environmental Effects of Poultry Meat Production in Terms of Sustainability

Environmental impact loads (climate change, eutrophication, acidification and energy requirement) in poultry meat production differ according to poultry production systems. The lowest values are in conventional, then free range and the highest in organic production system (Leinonen et al., 2012).

According to the results of the life cycle assessment, in the production of 1 kg of poultry meat, greenhouse gas emissions (kg CO_2 equivalent) occur in 0.40kg breeder, 0.16kg slaughterhouse and 0.12kg hatchery operations, with 1.68kg broiler rearing the highest share (Tetteh et al., 2022). In chicken meat production, feed and manure have the largest share of environmental impact burdens, such as climate change (greenhouse gas production), energy use and eutrophication, with 72, 62 and 60%, respectively. In acidification, the share of fertilizer is the highest with 65%. Similarly, Tetteh et al., (2022) reported that the share of feed in greenhouse gas emissions in chicken meat production is 70.7%, fuel 8.1%, wastewater treatment 4.1%, electricity 10.2%, enteric fermentation of manure 2.1%, transportation 3.2% and the share of other factors is 1.7%.

In the life cycle evaluation, it has been reported that the greenhouse gas emissions per kg body weight (in kg CO_2 equivalent) vary according to the countries, the production models of the countries, the growing period and the production years in the same country (Tetteh et al., 2022).

Feed and Nutrition Strategies in Sustainable Chicken Meat Production

In terms of feed and feeding strategies in sustainable chicken meat production, the preference of feed ingredients with lower environmental impacts, as well as the feed formulation that reduces the environmental burden due to chicken production factors in their use in feed also has an important share.

Use of sustainable feed ingredients: In terms of sustainability, environmental loads of feed ingredients can be different depending on countries, different locations and production practices within also the same country, and differences in post-production transportation etc. Based on feed production, the selection of better sustainable feed materials and lower environmental burden constitute the first step of feed production. In addition to traditional feed ingredients, alternative new feed materials with lower environmental burden have come to the fore with recent researches (Table 1). These alternative new feedstuffs have significantly lower greenhouse gas emissions and land area requirements than those of soybean meal. On the other hand, when the use of these new feed materials in poultry feeds is evaluated; these feedstuffs are produced mostly in very low quantities or on an experimental scale, the their production costs are high, the researches on the effects on broilers are generally experimental, the feeding value would be lower because of palatability and anti-nutritional factor issues, therefore it is necessary to carry out further research on these feed materials.

Table 1. Environmental loads, nitrogen and phosphorus contents of some alternative new feed ingredients and soybean meal (Tallentire 2018)

Feed ingredients	GHG (CO_2 equiv; kg/kg)	Land ocupation (m^2/kg)	Total N content (kg/kg)	Total P content (kg/kg)
Soymeal	3.05	3.11	0.075	0.006
Microalgae	2.31	0.034	0.093	0.014
Macroalgae	2.1	0.021	0.037	0.002
Duckweed	1.03	0.004	0.048	0.004
Yeast protein concentrate	1.08	1.26	0.108	0.013
Bacterial protein meal	1.49	0.026	0.117	0.015
Leaf protein concentrate	0.611	1.98	0.093	0.005
Insect meal	2.91	1.06	0.084	0.008

Reducing crude protein level in broiler feeds: Reducing the crude protein levels in broiler diets limits to supply some essential (EAA) and non-essential amino acids (NEAA) at the required levels; so, the addition of some feed crystal (synthetic) amino acids to diets become essential. In the implementation of this feeding strategy, especially in the use of feed ingredients other than corn and soy products, feed formulations in the digestible amino acid (SIDAA) concept and the ideal amino acid/ ideal protein concept should be adhered to as much as possible. Amino acid (AA) imbalances in mixed feed lead to higher uptake of some AAs in broilers, resulting in catabolism, cost of deamination, and possibly higher excretion of ammonia with feces, resulting in health problems.

Limiting amino acids may be different according to feed ingredients and their levels in broiler diets. While the 4th limiting AA in the corn & soybean meal based broiler diets is Valin (Val), Isoleucine (Ile) AA in case of 8% poultry by-product meal (PBM) inclusion, Tryptophan (Trp) in case of 3% PBM+3% meat-bone meal+2% feather meal inclusion in this diet. With the addition of the limiting amino acids such as Met, Lys, Thr and Ile or Val, the crude protein level in broiler grower diets can be reduced by up to 18.4% (Corrent and Bartelt 2011). Chrystal et al., (2020) reported that the crude protein level of the broiler feed can be reduced from 21.5% to 18.6% without any deterioration in performance. In parallel with the these reports, Belloir et al. (2017) indicated that reducing the crude protein levels from 19% to 16% by adding synthetic amino acids while maintaining the limiting amino acids levels in broiler feeds (21-35 days) demonstrated no significant effect on live weight and feed evaluation, an increase in breast meat rate, a significant increase in abdominal fat, a linear increase in pH and a decrease in brightness and drip loss as a meat quality parameter.

Reducing the crude protein level in broiler feeds increases nitrogen retention without adversely affecting technical performance parameters, and thus reduces nitrogen excretion into the environment with feces. In addition, it also reduces the water content of feces and the release of nitrogen into the environment by volatilization. Thus, the burden of feed production on climate change is reduced. Studies have shown that each 1% reduction in crude protein of broiler feeds reduces approximately 13% fecal nitrogen excretion, 30% airborne nitrogen excretion (Belloir et al., 2017) and 7% the climate change (CO_2 equivalent) burden of feed production per ton body weight gain (Cappelaera et al., 2021). Reducing the level of crude protein in broiler feeds also provides flexibility in the use of lower cost local feed ingredients and significantly reduces the use of high-quality protein sources such as soy products. The 1% crude protein reduction in the formulation of corn soy-based chicken diets based on SID AA and ideal AAs ratios concepts by supplementation of Met, Lys and Thr AAs results in approximately 4% less use of soybean meal (Cappelaere et al., 2021).

Reducing the crude protein level in broiler feeds has also positive effects on the health of the chickens. These positive effects are results of less undigested protein that reaches the last part of the intestine due to the supply of amino acids at the required level with low crude protein levels of feed and less the production of metabolites that occur with protein fermentation. As a result of all of these, less water is excreted with feces and the quality of the litter become better, as a result, the poultry environment and related poultry health are positively affected as well as the footpad dermatitis. It should not be forgotten that in addition to the positive results given above, animals that consume feeds with lower protein content at the required AAs levels are more tolerant to heat stress.

Use of feed additives that increase the utilization of nutrients including amino acids, phosphorus and other minerals: With the use of feed enzymes (phytase, carbohydrates and proteases, etc.) in broiler diets, the digestibility of amino acids is significantly increased and nitrogen excretion to the environment is reduced, as well as the reduction of phosphorus excretion

with feces to the environment by increasing the digestibility of phytate phosphorus. For other minerals, especially Cu, Se and Zn, the use of organic compound forms instead of inorganic salt forms also reduces the excretion of these minerals with feces and contributes positively to achieving an environmentally sustainable production due to their higher absorption.

Precision /multi-phase feeding: Smart feeding applications are also an alternative to reduce feed costs in feeding, reducing the excretion of nitrogen, phosphorus and other minerals excreted with feces and reducing the burden of environmental impact. Smart/multiphase feeding is based on the fact that the nutrient requirements of poultry will theoretically change daily. In the smart feeding, in each period, either two feeds are mixed daily at certain rates, or the two feeds produced during the initiation period are allocated to the animals by mixing the one that is higher in terms of energy and lower in terms of amino acid density with the other feed produced for the relevant period at certain rates on a daily basis until the end of the next feeding period. With this type of application, feed cost and nutrient waste are lower in production. In this method, at least two separate feed silos, a weighing scale, mixing system and bunker, as well as the target performance and nutrient requirements of the animals should be determined with accurate models.

Consideration of the environmental loads of feed ingredients and compound feeds in feed formulations: In the multi-purpose feed formulation (Eco ration), the environmental impact of the feed ingredients calculated by life cycle assessment (LCA) is taken into account. In some countries, there are databases and data sets (ECOALIM dataset, AGRIBALYSE database) created for this purpose (Wilfart 2016). These databases enable the calculation of acidification (H^+ equivalent/kg), climate change (kg CO_2 equivalent/kg), eutrophication (kg PO_4^{3-} equivalent/kg), energy requirement (MJ/kg) and area (m^2 years/kg) and phosphorus (kg P/kg) requirement parameters on LCA basis. In traditional poultry feed formulation (Least cost feed formulation, LCF), the approach is based on the nutrient costs of feed ingredients and does not take into account environmental factors. In eco-ration formulation, the environmental impacts of feed ingredients are included in the formulation and it is essential to optimize feed cost by minimizing environmental impacts by using the LCA database. Eco-ration tends to prioritize the use of locally produced feed ingredients in general to reduce the negative impact of the transportion of feed materials on the environment. In the formulation of Eco-ration, taking into account the produced geography of the feed ingredients, calculating the environmental impacts of the cultivation practices in the relevant area (whether the soil land used is opened by destroying the forest land, tillage, fertilization, irrigation and type, plant protection methods, etc.), drying, storage, national and international transportation status and the environmental impacts of a large number of items such as the production methods in the factory for transport vehicles, processing and feed additives and the inputs used, based on the country of production and the country of export/import, the status of the production area, port, storage/customs and feed factory are included in the system as calculated. In short terms, in traditional feed formulation, the lowest cost of feed production within the framework of nutrient and feed ingredient constraints is objective, besides this objective, in Eco ration, the feed production strategy is put forward within the framework of environment & economy with animal and animal production models as well as environmental impact and economic purposes. The environmental effects of feed ingredients calculated in different databases can take different values (Figure 1).

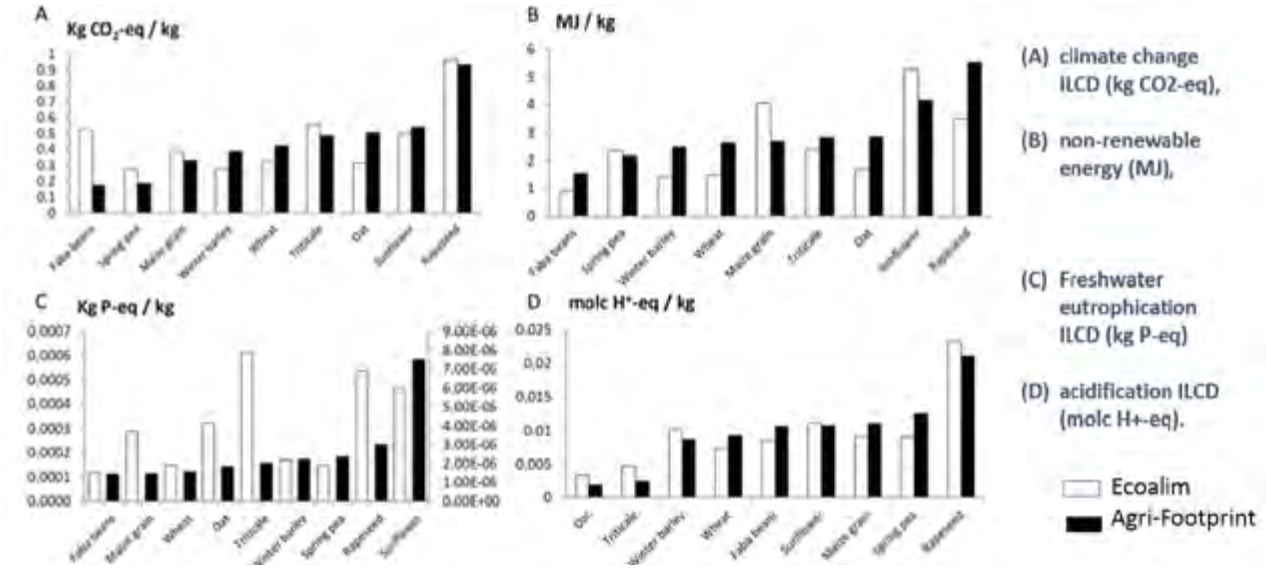

Figure 1. Comparison of impact assessment results per kg of feed ingredient for cereals, legumes, and oil and protein crops in the ECOALIM and Agri-Footprint databases (Modified from Wilfart et al. 2016)

In a study conducted by Meda et al., (2021) on broiler chickens, they formulated the broiler feeds based on both LCF and Eco-ration formulations at the same nutrient levels in starter diet with 2845 kcal ME/kg and 1.15% SID Lys; grower diet with 2940 kcal ME/kg and 1.05% SID Lys; finisher diet with 3010 kcal ME/kg and 0.93% SID Lys and considering the ideal AA concept according to SID Lys in all feeding periods. Significant differences were found between LCF and Eco-ration formulation for the parameters of environmental load effects per ton of live weight and feed price (Table 2). In the eco-ration-based solution, the environmental load values were generally lower at the feed mill and farm gates, while the feed cost was higher at the feed mill gate and lower at the farm gate. In contrast, since the nutrient contents of both feeds were kept the same, there was no technically significant difference in production parameters, waste and greenhouse gas emissions in the broiler house.

Conclusion

Although broiler meat production is in a better position than the production of other animal products in terms of sustainability, significant improvements can be achieved in the parameters of the environmental impacts of meat production by using strategies to reduce the crude protein content of feed in the lowest cost feed formulation. In addition to such feed and feeding strategies, in multi-purpose feed formulation programs (Eco ration), feed solutions to be made by taking into account the environmental impacts of the life cycle (LCA) of each feed ingredients can be used to reduce the environmental impact burdens of feed production in the feed mill gate and meat production at the farm gate, and sustainable broiler meat production. With the increase in consumer perception and preference, it is highly possible that important steps will be taken in sustainable broiler meat production and consumption with the introduction of environmental impact information on the packages of chicken meat and products as labels within the time period. As in all activities related to life, it should not be forgotten that sustainability is a journey in broiler meat production and that it is necessary to increase the target upwards by ensuring continuous data collection and data flow at every level reached, as well as making the evaluations correctly.

Table 2. Calculated environmental loads and feed costs of the least cost feed formulation (conventional, LCF) and multiobjective feed formulation (Eco-ration, MOF) solution-based broiler feeds at the feed mill and farm gate (Meda et al., 2021)

Feed Formulation method	Least-cost (LCF)	Multiobjective (MOF) (Eco ration)
Feed-mill gate (/t feed)		
Climate change (kg CO_2 eq)	827	727
Acidification (mol H^+)	11.9	11.5
Eutrophication (kg PO_4^{3-})	4.4	4.1
Cumulative nonrenewable energy demand (MJ)	7574	6179
Land occupation (m^2.yr)	1479	1538
Phosphorus demand (kg P)	7	6.2
Feed price (€)	294	304
Farm gate (/t live weight)		
Climate change (kg CO_2 eq)	1947	1777
Acidification (mol H^+)	52.7	51.9
Eutrophication (kg PO_4^{3-})	12.5	12
Cumulative nonrenewable energy demand (MJ)	20595	18240
Land occupation (m^2.yr)	2860	2959
Phosphorus demand (kg P)	12.3	10.8
Feed price (€)	152	136

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OP03 Use of Medium Chain Fatty Acids, Phytogenics and Polyphenols in Broiler Chickens Diets

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Abstract

The objective of this experiment is to evaluate the MCFA or MCFA with phytobiotics (thymol, saponins and tannins) or polyphenols on the performance, carcass yield and anti-inflammatory parameters as well as TBARS in broilers. This trial was carried out with 528, 1-day-old male Ross 308 chicken broilers in 4 treatments with 12 replicates per treatment and 11 birds per replicate. The total trial duration was 40 days with a 3-phase feeding system (starter 1-10d), grower (11-24d) and finisher (25-until end). The dietary treatments were as follows: T1 - control, T2 -MCFA and T3 MCFA+FITO, supplemented with correspondingly Aromabiotic Poultry (MCFA - C6, C8, C10, C12) or M-prove (MCFA - C6, C8, C10, C12 and thymol, saponins and tannins) in doses of 1.2 kg per ton of feed in starter and grower and 0.7 kg/ton in finisher feed. T4 – Phenols, were supplemented with Vitanox – polyphenols. The use of MCFA or MCFA+FITO improved FCR and BWG in comparison with the Control treatment (1-40d, $P<0.05$). Birds fed diets with polyphenols (Phenols) grow better than Control ($P<0.05$) but consume more feed which results in similar FCR (Control vs. Phenols). Use of MCFA, MCFA+FITO or Phenols decreased fats peroxidation products in breast muscles in comparison with Control birds ($P<0.05$). Birds fed MCFA+FITO had higher IL1, IL6 and lowered IL8 blood concentrations in comparison with the Control ($P<0.05$). Similar differences ($P<0.05$) were observed for jejunum tissue IL's concentration. Additionally, TNF in blood serum and ileal tissue didn't change across treatments. In our study, MCFA didn't change ILs concentration.

Introduction

Alternatives for antibiotic growth promoters, as well as natural antioxidants, are of the interest poultry industry. Recent studies have demonstrated that dietary supplementation of different medium-chain fatty acids (MCFAs) improves birds' performance [1]. Some studies showed the significant role of dietary MCFAs in the regulation of intestinal health in animals [2]–[4]. Current literature data showed that phytobiotics like thymol, saponins and tannins are reported as an effective alternatives for antibiotics growth promotors and anticoccidial agents [5]–[7]. The supplementation of poultry diets with antioxidants has been implemented to achieve optimal growth performance and meat quality [8], [9].

The objective of this experiment is to evaluate the MCFA or MCFA with phytogenic molecules (thymol, saponins and tannins) or polyphenols on the performance, carcass yield and anti-inflammatory parameters as well as TBARS in broilers.

Materials and Methods

This trial was carried out with 528, 1-day-old male Ross 308 chicken broilers in 4 treatments with 12 replicates per treatment and 11 birds per replicate. The total trial duration was 40 days with a 3-phase feeding system (starter 1-10d), grower (11-24d) and finisher (25-until end). The dietary treatments were as follows: T1 - control, T2 -MCFA and T3 MCFA+FITO, supplemented with correspondingly Aromabiotic Poultry (MCFA - C6, C8, C10, C12-total 60% of MCFA) or M-prove (MCFA - C6, C8, C10, C12 and thymol, saponins and tannins, total of 44,75% of MCFA and phytogenics) in doses of 1.2 kg per ton of feed in starter and grower and 0.7 kg/ton in finisher feed. T4 – Phenols, was supplemented with Vitanox – polyphenols (min. 11% of polyphenols). On day 40, 96 chickens (2 birds/pen, hence 24 birds/treatment) were randomly selected and euthanised by electric stunning following the recommendations for the euthanasia of experimental animals. Then, 96 blood samples needed for further analysis were collected. Ten blood serum samples were obtained by centrifugation for 10 min at 3500 × g, then frozen at −80°C until further analysis. Ten left breasts per treatment (40 in total; *pectoralis major*) were collected for drip loss. Additionally, the right breast (10 per treatment, 40 in total) was collected for TBARS determination. Thiobarbituric Acid Reactive Substances (TBARS) were determined using colorimetric assays based on TCA Method. TBARS were determined on 60 samples using Cayman's TBARS (TCA Method; assay kit – 700870). For drip loss,

breast muscles were weighed post-mortem, suspended in transparent bags with holes for water leakage, and after 48-h of storage at 2 °C, were weighed again [10]. The levels of interleukins (IL-1, IL-6, IL-8, and IL-12), and tumor necrosis factor-alpha (TNF-α) were determined by using commercially available enzyme-linked immunosorbent assays (ELISA) according to manufacturer instructions using blood and tissue samples obtained at the indicated stage of the experiment. In brief, tissue fragments were cleaned of intestinal contents and taken into liquid nitrogen immediately after the experiment. Samples were preserved for future analysis at -80°C. After this, a small piece of tissue was homogenized on ice in an appropriate buffer (RIPA or PBS), and then the homogenate was centrifuged. The supernatant was collected for analysis. Absorption values of ELISA and colorimetric tests were taken using the Synergy 2 microplate reader from Biotek (USA).

The pen was the experimental unit, $P<0.05$ was considered significant, and $P<0.1$ was a trend. The experiment was conducted as a completely randomized design. Growth performance, MDA or ILs concentration were analysed by analysis of variance (ANOVA) using the general linear models procedure of SAS (9.4).

Results and Discussion

Data are presented in table 1. The use of MCFA or MCFA+FITO improved FCR and BWG in comparison with the Control treatment (1-40d, $P<0.05$). Birds fed diets with polyphenols (Phenols) grow better than Control ($P<0.05$) but consume more feed which results in similar FCR (Control vs. Phenols). Previous studies [1], [2] have demonstrated that the use of different medium-chain fatty acids (MCFAs) protected the gut function in broilers against necrotic enteritis challenge, intestinal inflammatory responses and immune function, which also subsequently translated into better nutrients digestibility and finally performance results. In the presented study, birds were not challenged. However, we used diets with 5 % in starter or 10 % (in grower and finisher diets) inclusion of rye to increase digesta viscosity and create more challenging conditions for nutrient absorption. This should result in a higher proliferation of microbes. Buyse et al., (2021) reported that birds-fed saponins (in low concentration) were characterised by better performance. This was attributed to better enterocyte proliferation, which resulted in a better absorptive surface of the digestive tract. Poultry meat is pretty rich in unsaturated fatty acids and is, consequently, susceptible to oxidative deterioration. This process is recognized as a negative for meat's nutritional quality and flavor [13]. There were no differences in carcass breast muscle per cent as well as drip loss (48h) across treatments (data not presented). Use of MCFA, MCFA+FITO or Phenols decreased fats peroxidation products in breast muscles in comparison with Control birds ($P<0.05$). It can be speculated that saponins present in M-prove decreased the degradation of fats in chickens' breast muscles, which resulted in a decrease in MDA ($P<0.05$). Chi et al., (2017) reported that tea saponins significantly improved antioxidant activity, as indicated by decreasing MDA.

Birds fed MCFA+FITO had higher IL1, IL6 and lowered IL8 blood concentrations in comparison with the Control ($P<0.05$). Similar differences ($P<0.05$) were observed for jejunum tissue IL's concentration (data not presented). Additionally, TNF in blood serum and ileal tissue didn't change across treatments (data not presented). In our study, MCFA didn't change ILs concentration. Sacakli et al., (2023) reported that MCFA could decrease the relative mRNA abundance of IL-1b, IL-10, TNF-a, and IL-17A in the jejunum of birds. Above mentioned authors speculated that it might suggest the anti-inflammatory effect of MCFA.

Conclusion

It can be concluded that both MCFA and phytobiotics can improve birds' performance. Additionally, all tested additives (MCFA, MCFA + phytobiotics and polyphenols) can enhance poultry meat quality and stability.

Table 1. Growth performance (BWG, FI and FCR), breast muscles MDA and blood IL concentration in birds fed Control, MCFA, MCFA+FITO or PHENOLS diets from 0-40d.

	Control	MCFA	MCFA+FITO	Fenoller	*SEM*	*P*
	1-10					
BWG	273b	282a	276ab	280a	1.260	0.03
FI	277a	274a	266b	278a	1.274	0.003
FCR	1.017a	0.9712bc	0.9640c	0.9912b	0.0045	<0.001
	11-24					
BWG	913	915	912	899	5.52	0.752
FI	1304	1265	1257	1236	9.082	0.710
FCR	1.429	1.383	1.379	1.374	0.0076	0.980
	25-42					
BWG	1943b	2060a	2099a	2068a	15.07	<0.001
FI	4035	3994	4028	4199	35.51	0.172
FCR	2.078a	1.943bc	1.921c	2.03ab	0.019	0.009
	1-42					
BWG	3128a	3257b	3287b	3247b	17.47	0.005
FI	5615	5533	5551	5712	38.8	0.362
FCR	1.795a	1.700b	1.689b	1.759a	0.0117	0.002
	Breast muscles:					
MDA	53.92a	37.03b	20.52b	30.11b	3.322	0.002
uM MDA/mg protein	3.98a	2.69b	1.43b	2.23b	0.251	0.002
	Blood Serum:					
IL1 [pg/ml]	39.13bc	43.77ab	47.86a	32.04c	1.559	0.001
IL6 [ng/L]	61.89b	57.06b	86.54a	64.71b	3.065	0.002
IL8 [ng/L]	98.64a	80.73ab	68.75b	99.07a	4.490	0.038

SEM – pooled standard error of the mean, ^{abc} – P<0.05

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OP04 *Tenebrio molitor L.* larvae Inclusion in Quail Diet as Fat Source: Effects on Performance, Meat Quality and Fatty Acid Composition

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Abstract

This study was conducted to determine the effects of *Tenebrio molitor* (TM) larvae oil inclusion in diets instead of soybean oil and fish oil on performance, carcass and meat quality of Japanese quail. A total of 180 10-day-old quail (*Coturnix japonica*) were weighed and divided into 15 cages for three treatment groups. The dietary treatments were as follows: 1) control diet (C) formulated with reference to the conventional diet (SBM based diet) 2) a fish oil diet (FO) which was formulated with fish oil supplementation as 30 g/kg and 3) a yellow mealworm (*Tenebrio Molitor* larvae) oil diet (TMO) which was formulated with yellow mealworm supplementation as 100 g/kg in diet (contains 30 g/kg oil). No differences were found daily weight gain and daily feed intake ($P>0.05$). Final body weight was higher in quail fed TMO and lower in quail fed FO ($P<0.01$). Feed conversion ratio was improved with TMO diet in quail ($P<0.05$). Carcass weight was higher in TMO group than FO group ($P<0.05$). No differences were found in liver, heart, and spleen percentages between treatment groups ($P>0.05$), however proventriculus percentage was higher in FO compared to TMO group. Chemical characteristics of breast meat were unaffected by dietary treatments ($P>0.05$). The fatty acids were mostly similar in quail breast meat fed C and TMO diets however PUFA was lower in TMO diet compared the C diet ($P>0.05$). The FO groups C14:0, C20:4 n6 and n-3 fatty acids (C18:3, C20:3, C20:5, C22:6), SFA, PUFA and n-3/n-6 were increased, MUFA, PUFA n-6 and n-6/n-3 were decreased compared the C and TMO groups ($P<0.01$).

Keywords: mealworm, meat quality, fish oil

Introduction

Fat sources used in poultry nutrition mainly consist of oils that are not used in human nutrition (rendered fats from slaughterhouse) and vegetable oils. Soyabean meal (SBM) and fish meal are feed ingredients used as the main protein and energy source for poultry diets [1]. However, in recent years, limited supply and high prices of soybeans have led to increased interest in seeking new alternative lipid sources for poultry feed.

Insects are high-quality, efficient, sustainable, and affordable sources of protein [2]. The SBM is widely used as animal feed due to its protein content and quality [3]. In addition, insects are an important source of fat, like protein source [4]. Using insect meal and soybean oil as energy sources can prevent the use of large plantations and overfishing [5, 6]. Because soybean cultivation requires large areas and therefore it is known to cause more damage to the environment [7, 8]. Due to the increased demand for protein in the world and new trends in food preferences, more poultry meat is produced [9]. The increase in the demand for animal feed due to the increase in production may put pressure on the limited feed resources [10]. Especially the use of high amounts of soybean and fish meal as feed makes the resources limited [11]. Due to the limited amount produced and the high prices, alternative searches have emerged for poultry feed [12]. In fact, until now, interest in insects as food has mainly focused mainly on their protein content and quality [13]. Some insect species are fatty and in larval stages has higher fat than in adults [14]. In addition, lipids are also an important component of insects and are produced during the separation of proteins. The importance of degreasing insect meals, using insect protein concentrates as animal feed ingredients, and using lipids in animal diets and biodiesel production. Therefore, fat removal is recommended to improve the shelf life of the diet and to increase the protein digestibility of insect diets. Some edible insect species are known as a source of amino acids and phosphorus [15]. *Tenebrio*

Molitor (TM) is an unwanted pest in food industry [16]. However, TM is one of the insects that can be used in poultry feed due to its nutritional components and ease of cultivation [17]. *Tenebrio molitor* may contain 25-60% crude protein and 15-40% fat in dry matter [18, 19]. In addition, Tenebrio Molitor contains high amounts of zinc, selenium, biotin, pantothenic acid, folic acid, and unsaturated fatty acids, similar to fish meal and SBM [5, 20, 21]. In addition, TM fat has a high linoleic

acid content and is considered a source of n-6 polyunsaturated fatty acids [18].

Quail is considered the smallest poultry used in meat production. Since quail is the smallest animal used for poultry meat production, a large number of birds manage easily and can be housed in a small area [22]. Quail meat plays an important role in human nutrition due to its protein content and quality [23]. Quail meat is preferred because it contains less cholesterol, and saturated fatty acids and fat than sheep and beef [24, 25]. There is a connection between the amount and type of raw materials used in the ration and meat quality and carcass characteristics [22]. Quail meat quality may affect genetic and environmental factors [25]. There is a relationship between the energy source it receives from the diet and the quality of quail meat [22].

Currently, little is known about suitability of the TM larvae in poultry dietary ingredient as fat, and to the best our knowledge, no studies have determined the effect of replacing soybean and fish oil with TM larvae on performance and meat quality. Therefore, the aim of this study was to evaluate the effect of dietary fat from TM larvae on quail growth performance, carcass characteristics and meat quality.

Materials and Methods

Experimental design and feed preparation : A total of 180 7-day-old quails (Coturnix coturnix japonica) were housed with both sexes in battery packs in a controlled environment room. Chicks were divided into 12 to 15 cages (five replicates per treatment) and received three dietary treatments until slaughter age: control diet (C) formulated with reference to the conventional diet (SBM based diet) used by the breeder on the farm, a fish oil diet (FO) which was formulated with fish oil supplementation as 30 g/kg and a yellow mealworm (*Tenebrio Molitor* larvae) oil diet (TMO) which was formulated with yellow mealworm supplementation as 100 g/kg in diet (contains 30 g/kg oil). Diets were formulated as isocaloric and isonitrogenous and formulated according to the NRC [26] to meet requirements of Japanese quails. The experimental diets' ingredients and chemical composition are provided in Table 1. Feeds and water were provided ad libitum. Light was applied 16 hours a day. The average minimum and maximum room temperature and humidity levels were recorded weekly.

Chemical composition analysis : The meat obtained from the right breast was weighted and freeze-dried at −40°C for chemical composition analyses. The dried meat grounded to obtain a fine powder and analyzed for moisture, protein, and ash [27]. Fat content was calculated the difference of protein and ash from moisture [28].

Growth performance and slaughtering procedures : Mortality rates were recorded daily. Body weight and feed intake of the birds were recorded at the beginning and end of the experiment. The body weight gain (BWG) and feed conversion ratio (FCR) from 7 to 35 days of age. At 35 days of age, 3 males per replicate (15 per treatment group) were selected randomly and weighed the live weights. The birds were slaughtered and after the feathers were removed, the entire digestive tract, head, neck and legs were weighed to determine the percentage of carcass. Intestines and internal organs (liver, spleen, gastric glands and heart) were removed and weighed; the results were expressed as a percentage of carcass weight.

Meat quality parameters **:** The pH measurements were performed using a pH meter (Mettler Toledo, Milan, Italy) at 2 different sites on the raw breast meat of each sample. The obtained values were considered as 2 replicates and their average was used. Measure the amount of drip in raw breast samples (in triplicate) at 4 °C. To determine the drip losses meat samples were reweighted after the refrigerated storage period (24 hours) and calculated as percentage of weight lost [29]. All measurements were performed on raw meat at room temperature. The meats were boiled (at 80 °C) and samples were reweighted after cooking for cooking loss. The obtained values were considered as 2 replicates and their average was used. The meat colour was measured using a colorimeter (Konica Minolta CR400, Osaka, Japan) according to the CIE Lab system. The lightness (L*) is represented on a scale from 0 to 100 from black to white, red values (a*) range from red (+60) to green (-60), and yellow values (b*) Ranges from yellow (+60) to blue (-60).

The method was modified [30] to recover the total lipids from breast meat. The fatty acid (FA) profile was examined using the isolated lipids. The FAME (Fatty Acid Methyl Ester) analysis was carried out by gas chromatography using a modified method [31]. The fatty acids were determined by FAME retention time were compared with the typical component FAME mix (Supelco, Bellefonte, PA, USA). The tricosanoic acid (C23:0) (Supelco) was used as an internal standard and obtained the calibration curves for fatty acids measurements. The fatty acids concentrations were expressed as g/100 g of sample

The FA profile was evaluated for human nutrition and health, the sums of the total saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA(PUFA), n–6 and n–3 were calculated, as well as the n–3:n–6 and n–6:n–3 ratios. In addition, the lipids nutritional quality was evaluated for the thrombogenicity index (TI) and atherogenicity index (AI) according to Ulbricht and Southgate [32]. The relationship between hypercholesterolemic and hypocholesterolemic FA (h:H) were calculated according to the Santos-Silva et al. [33].

Statistical analyses : Data were analyzed by One way ANOVA using the GLM procedure of SPSS (IBM SPSS Statistics 22), according to the model

$$Yij = m + OSi + eij,$$

in which Y is that the single observation, m is that the general mean, OS is that the effect of oil source (i = FO or TMO), and e is that the error. Variations among suggests that were separated exploitation Tukey's test at $P < 0.05$. The replicate was thought of the experimental unit.

Results

Table 2 shows the effects of FO and TMO inclusion on the performance of quail. No differences were found in initial body weight, daily weight gain and daily feed intake ($P>0.05$). Final body weight was higher in quail fed TMO and lower in quail fed FO ($P<0.01$). Feed conversion ratio was improved with TMO diet in quail ($P<0.05$). Effects of dietary FO and TMO inclusion on carcass and internal organ weights percentage of carcass weights of quail are shown in Table 3. Carcass weight was higher in TMO group than FO group ($P<0.05$). No differences were found in liver, heart, and spleen percentages between treatment groups ($P>0.05$), however proventriculus percentage was higher in FO compared to TMO group. Table 4 shows the meat quality parameters of slaughtered quail. Only muscle b* was increased in quail fed C and FO than in quail fed TMO ($P<0.05$). Chemical characteristics of breast meat were unaffected by dietary treatments ($P>0.05$). The FA composition of the quail breast meat of the experimental groups are reported in Table 5. The fatty acids were mostly similar in quail breast meat fed C and TMO diets however PUFA was lower in TMO diet compared the C diet ($P>0.05$). The FO groups C14:0, C20:4 n6 and n-3 fatty acids (C22:6, C20:3, C20:5, C18:3), SFA, PUFA and n-3/n-6 were increased, MUFA, PUFA n-6/n-3 and n-6 FA were decreased compared the C and TMO groups ($P<0.01$).

Discussion

Performance : Feed intake was not different between treatment groups, however FBW and FCR were improved with TMO fed quail. In present study, TM larvae was administrated at 10% level which level was preferred to obtain 3% oil from TM (contains 30% crude oil) in diet. In previous studies TM larvae were supplemented from 0.5% [34] to 29.6% level [35]. When the TM larvae supplemented to quail diets more than 2%, the body weight and gain, and FCR of the quail improved, however feed intake did not affect [36]. Bovera et al. [35, 37] reported that feed intake and FCR were improved in fast-growing chickens fed dietary SBM fully replaced (29.6%) with TM meal. The FCR was a decreasing trend in broiler fed with TM from 0% to 10% of inclusion in the diet [34]. On the contrary, the growth performance was unaffected in intermediate-growing chickens fed the TM meal supplemented diet at 7.5% [38]. In the present study showed that dietary fish oil supplementation (3%) decreased the final BW of quail. In a study showed that fish oil supplementation did not effect the performance of broiler, however, there was a negative effect of the fish oil on the final weight and FCR of broilers at the finisher phase [39].

Carcass and meat quality **:** In our study, the carcass weight and proventriculus incidence on carcass weight was decreased with FO inclusion, but not affected with TM inclusion to the diet in quail. In a study, there was no significant difference in carcass characteristics with TM supplementation or control diet in quail [36]. The carcass characteristics did not effect of dietary TM inclusion of the birds [40]. The results are in agreement with Biasato et al. [41] and Bovera et al. [37] who showed that dietary TM meal did not affect the slaughtering performance of broiler chickens. In contrary to Ballitoc and Sun [34] 2% inclusion TM larva meals to diet were improved slaughter weights and yield, dressing percentage in broiler chicken. Loponte et al. [42], found an improvements of carcass weights fed in replaced with 25 and 50% of the SBM with TM larva meal in diets. Biasato et al. [43] reported that the carcass weight, abdominal fat weight and abdominal fat percentage were increased in female broiler chickens fed with TM larva meal partial substitution of soybean oil, corn gluten meal and SBM. Dietary TM meal supplementation of different levels (1, 2 and 3 g/kg of diet) in broilers, carcass yield was improved in treatment groups compared to control group [44].

The pH_{24} values of breast muscles were not changed significantly by treatment. When breast meat pH_{24} values lower than 5.7 the broiler breast meat can be classified as soft, pale and exudative. The pH higher than 6.2 broiler breast meat can be classified as dark, firm and dry [45]. In the current study, pH_{24} values (6.17-6.27) of breast muscles were higher than a study that breast muscles were ranged at 5.72-5.77 with dietary TM inclusion at different levels (1.65, 3.3. and 6.6 g/100g) on quail diets [46]. This differences related to animal genotype, species, and rearing system [47]. This differences also related to the dietary factors (such as TM levels; low molecular-weight nitrogenous compounds) and related to the digestive tract's metabolism that could affect the initial pH [48]. Our results are in contrast with those reported by no difference in breast muscle pH among fed with or without TM [40, 46]. In present study, the meat color parameters were not affected with

dietary treatments, however the b* color value was decreased in TMO group (P<0.05). Zadeh et al. [49] reported that any significant difference in on 35-day-old Japanese quails breast meat L* value was lower (43.09–43.41) as compared to our study. The consumers are more preferred lighter breast meat color than dark meat color [50]. The results of L* (Lightness), a* (redness), and b* (yellowness) are not in agreement with Bovera et al. [37] found that the breast meat L* was (44–44.2), a* was (1.07–1.18), and b* was (0.69–0.78). Our results are in contrast with Zadeh et al. [49] reported by a decrease in b* fed with increasing the level of TM in Japanese quails. Moreover, the broiler breast meat color can vary due to the meat pH, in a study raw meat color has a significant linear relationships between and its pH [51].

The breast meat proximate composition (moisture, ash, protein and fat) was not affected by the dietary treatment. Birds slaughtered on 35 days of age and had the same moisture (72.45–72.75%), protein (24.00–24.96%), fat (1.48–1.98%), and ash content (0.99–1.04%) in breast meat. It has been reported that the absence of feeding-related differences in the approximate composition of meat is an important finding for the positive evaluation of insects as a new alternative feed in poultry nutrition [40]. Similar to our results, Bovera et al. [37] found no significant effect on the approximate composition of meat from broiler breasts fed diets containing TM larva meal during the growth period. Differently, Ballitoc and Sun [34] added different levels of TM meal to the broiler diet (0.5, 1, 2, and 10%), showed that the addition of 1% TM showed the lowest moisture percentage and higher protein content in breast meat compared to the other groups.

In the present study, the breast meat drip, cooking, and thawing losses were not affected by the dietary treatments. Meat losses were not statistically affected, even though they were higher in the TMO group. WHC or drip loss indicates the maximum moisture level that the muscle can hold [49]. In addition, the high pH of the meat also affects drip loss [52]; poultry meat has a low pH and low WHC, which reduces cooking loss, drip loss and shelf life [53]. In a study, the effect of adding TM to the diet (5 and 10%) on breast meat cooking loss was found to be insignificant [54]. In contrast, Bovera et al. [37], cooking loss was lower in the TM group than in the SBM group. Cooking loss is calculated as the percent weight difference between fresh and cooked samples according to the weight of fresh meat samples [55]. This loss is an important indicator of meat quality as it determines the technological yield of meat [56]. Therefore, it is thought that the use of TM in the diet does not change the quality of meat.

Meat fatty acid composition : In the current study, a diet based on SBM, which is the most preferred vegetable source in poultry nutrition, and fish oil was formulated and compared with a diet supplemented with 30% fat mealworm larvae. Additionally, this is the first study that, to the best of the authors' knowledge, compares feeding quail meals with TMO to feeding them foods with FO. Even though the two groups of broilers in the current study received diets with equal amounts of lipids, the main source of fat (corn and SBM) in the control group's diet was vegetable oil, while the main source of fat in the TM group's diet was full-fat TM larvae meal, which contained about 30% ether extract and therefore no vegetable oil. The breast meats predominant fatty acids of quail-fed both the control and TM diets was C18:1 c9 (29.71 and 35.55 g/100 g total FA) followed by C16:0 (23.27 and 24.72 g/100 g total FA) and C18:2 n-6 (15.81 and 17.93 g/100 g total FA). The TMO group were similar with C group in C18:1 c9, C16:0 and C18:2 n-6 fatty acids, however C18:3 n-3 was increased in TMO group compared to the C group (P<0.05). In a study, TM supplementation showed higher C18:1 c9 and C18:3 n-3 [40], and contemporarily lower SFA and C16:0 concentrates in breast meat. Similarly oleic acid (C18:1 c9) is the predominant fatty acids [57] in meat and increased in TM larvae. In present study, quail breast meat fatty acid composition was very similar with a study results used in full-fat TM larva meal reported by Kierończyk et al. [4].

The TM larvae oil-based diet (TMO) group breast meats were not shown a significant differences compared to the SBM based diet (C) group. However, despite the large differences in the FO diet, especially in the major fatty acid groups (SFA, MUFA, PUFA), there was no difference between breast meat from the two diets (C and TMO) treatments. In quails fed with three different diets, there was no significant difference in breast meat fatty acid composition, C (SBM based diet), and TMO (TM larvae-based diet), but significant changes were observed in FO fed quail meat fatty acid composition. A higher level of C14:0 was detected in quail breast meat fed on a diet supplemented with FO, but no change in breast meat in quail fed on a TM or C diet. In the previous study, C14:0 concentration was increased in breast meat in broilers fed with a full-fat TM containing diet [58]. No differences were observed for SFA and MUFA between the breast meat from C and TMO treatments; however, PUFA were decreased in TMO group. Kierończyk et al. (2018) observed that SFA decreased and MUFA increased in breast meats of TM oil-fed chickens, but the PUFA level of SBM-fed chicken breast meat did not change. In contrast, PUFA fatty acids were higher in the meat of broiler chickens fed the TM larval diet [59]. Even if the same insect species are used, there is inconsistency between the results of other experiments, which can be attributed to the variability of the fatty acid composition of insect oil due to the nutrients used in larvae production [60].

In this study, the FO group significantly affected the fatty acid composition of the meat. ω-3 FAs were increased in the FO

group. Similar to the findings of this study, Shin et al. [61] showed that feeding broiler chicken with fish oil can cause an increase in ω-3 FA content and a decrease in ω-6 FA in chicken meat. This can be explained by the competition between the ω-3 and ω-6 FA families, since the transformation of ω-3 and ω-6 FAs share the same type of enzymes, so an excess of one over the other indicates a significant reduction of the other [62]. Shin et al. [61] reported that ω-3 FA (EPA and DHA) content increased in meat of chickens fed a diet rich in fishmeal or fish oil. A recommendation by the UK Department of Health is that the PUFA/SFA ratio should be >0.45 and the ω-6/ω-3 ratio <4.0 in human diets [63]. In this study, all treatments of PUFA/SFA ratio met these recommended levels, but only FO treatment met the recommended ratio level of ω-6/ω-3. Some indices (AI, TI) that relate different amounts of some specific fatty acids are calculated and used to show the contribution of these fatty acids to the prevention or support of some pathological phenomena in the human diet [64]. The current study showed that the TMO group had significantly lower AI in breast meat compared to the FO group, suggesting that both groups had lower AI values and could be considered healthy for human consumers [65]. Loponte et al. [58], did not observe a difference in breast meat quality indices (PUFA, AI, n6/n3 ratio and TI) of SBM supplementation with the addition of TM larvae meal to the diet of broiler chickens.

Conclusions

Based on results of the present trial, TM larvae can be considered a feedstuff as SBM. However, TM supplemented diets have no potential for more healthy fatty acid profile namely low in SFA and high in PUFA, and with a favorably low n-6/n-3 ratio like FO supplemented diets. In addition, considering that these results may vary depending on the rearing factor of the larvae; It is very important to feed the larvae for the production of better quality meat from animals that feed on these insects.

Ethics Statement

The experiment was carried out in ERUTAM (Erciyes University Research and Application Center), after the approval by the local animal ethical committee (ERUHADYEK) with the 22/121 number.

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Table 1. Dietary ingredients and chemical composition of the experimental diets

Ingredients, g/kg DM	C	FO	TMO
Corn	563.7	528.0	569.8
Soybean meal	385.8	390.0	300.9
Gluten meal	21.5	23.4	-
Fish oil	-	30.0	-
Mealworm larvae	-	-	100.0
Limestone	13.8	13.8	12.9
Salt	4.2	3.5	4.0
Vitamin and mineral premix[1]	2.5	2.5	2.5
L-Lysine	1.2	0.3	0.4
Dicalcium phosphate	7.0	7.0	8.5
L-Threonine	0.3	1.5	1.0
Composition			
DM, g/kg	899.7	900.1	900.6
CP, g/kg	240.0	240.0	240.0
ME, kcal/kg	3000	3000	3000
Ca, g/kg	8.0	8.0	8.0
Available P, g/kg	3.1	3.0	3.1
Na, g/kg	2.0	1.9	1.9
Cl, g/kg	2.9	2.8	2.8
Met+Sis, g/kg	8.4	8.3	8.4
Lysine, g/kg	13.6	13.5	13.5
Threonine, g/kg	10.4	10.4	10.3
Tryptophane, g/kg	3.3	3.3	3.3
EE, g/kg	18.5	47.2	47.2
CA, g/kg	58.5	57.6	57.8
CC, g/kg	27.7	27.1	27.7
Starch, g/kg	390.4	390.8	389.7

C: control; FO: fish oil supplementation (3%); TMO: *T. molitor* larvae supplementation (10% for 3% oil) DM: dry matter; CP: crude protein; ME: metabolizable energy; EE: ether extract; CA: crude ash; CC: crude cellulose [1]Mineral-vitamin premix includes per kg of diet: retinol acetate, 4500lg; cholecalciferol, 50 mg; tocopherol acetate, 40.0 mg; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; pyridoxine, 5.0 mg; cobalamin, 0.03 mg; nicotinic acid, 30.0 mg; biotin, 0.1 mg; calcium d-pantothenate, 12 mg; folic acid, 1.0 mg; choline chloride, 400 mg; manganese, 80.0 mg; iron, 35.0 mg; zinc, 50.0 mg; copper, 5.0 mg; iodine, 2.0 mg; cobalt, 0.4 mg; selenium, 0.15 mg assured.

Table 2. Effects of dietary fish (FO) and Tenebrio Molitor larvae oil (TMO) inclusion on the performance of Japanese Quail

	C	FO	TMO	SEM	P
Initial BW (g, day 7)	67.61	63.60	67.63	0.480	0.747
Final BW (g, day 35)	204.43b	195.11c	212.28a	1.310	0.000
Daily weight gain, g	4.89	4.70	5.17	0.854	0.075
Daily feed intake, g	15.32	14.98	15.45	0.21	0.337
Feed conversion ratio, 7-35 d	3.16b	3.22b	3.02a	0.03	0.049

C: control; FO: fish oil supplementation (3%); TMO: *T. molitor* larvae supplementation (10% for 3% oil)

Table 3. Effects of dietary fish (FO) and Tenebrio Molitor larvae oil (TMO) inclusion on carcass traits and internal organs weight of Japanese Quail

	C	FO	TMO	SEM	P
Carcass weight, g	143.19ab	132.98b	151.62a	2.250	0.015
Liver, %	3.59	3.11	3.24	0.136	0.344
Heart, %	1.31	1.18	1.32	0.025	0.064
Spleen, %	2.54	2.54	2.70	0.065	0.463
Proventriculus, %	0.66ab	0.69a	0.61b	0.012	0.036

C: control; FO: fish oil supplementation (3%); TMO: *T. molitor* larvae supplementation (10% for 3% oil)

Table 4. Effects of dietary fish (FO) and Tenebrio Molitor larvae oil (TMO) inclusion on meat quality parameters of Japanese Quail

Parameters	C	FO	TMO	SEM	P
pH	6.17	6.17	6.27	0.020	0.085
Color					
L*	47.10	47.82	47.75	0.474	0.815
a*	11.20	11.55	11.20	0.291	0.489
b*	7.74a	7.98a	5.30b	0.366	0.001
Moisture	72.75	72.76	72.45	0.339	0.621
Crude protein	24.25	24.77	24.96	0.176	0.075
Crude ash	1.02	0.99	1.04	0.078	0.102
Ether extract	1.98	1.48	1.55	0.051	0.084
Cooking loss	25.63	26.87	26.38	0.461	0.565
Thawing loss	3.53	4.41	4.91	0.384	0.357
Drip loss	2.48	2.43	2.80	0.258	0.826

C: control; FO: fish oil supplementation (3%); TMO: *T. molitor* larvae supplementation (10% for 3% oil)

Table 5. Effects of dietary fish (FO) and Tenebrio Molitor larvae oil (TMO) inclusion on meat fatty acid composition of Japanese Quail

Fatty acids, g/100 g FAME	C	FO	TMO	SEM	P
C14:0	0.57b	1.42a	0.63b	0.07	0.000
C16:0	23.27	24.72	24.18	0.39	0.342
C16:1	8.30ab	6.67b	8.85a	0.37	0.041
C18:0	8.82	9.87	8.63	0.30	0.213
C18:1n9c	33.09a	29.71b	35.55a	0.63	0.000
C18:2n6c	17.93a	15.81b	16.41ab	0.37	0.065
C20:0	0.69a	0.68ab	0.55b	0.02	0.026
C18:3n6	0.19b	0.29a	0.31a	0.02	0.002
C18:3n3	2.24b	6.77a	0.19b	0.64	0.000
C20:3n3	0.20b	1.65a	0.34b	0.16	0.000
C20:3n6	3.89a	0.77b	3.52a	0.31	0.000
C20:4n6	0.31b	1.39a	0.29b	0.10	0.000
C20:5n3	0.27a	0.11b	0.24a	0.01	0.000
C22:6n3	0.24ab	0.15b	0.29a	0.02	0.004
Quality indexes					
SFA	33.34b	36.68a	34.01ab	0.57	0.040
MUFA	41.39a	36.38b	44.40a	0.90	0.000
PUFA	25.26a	26.93a	21.59b	0.71	0.002
PUFA n-6	4.39a	2.45b	4.13a	0.24	0.001
PUFA n-3	2.95b	8.67a	1.05b	0.72	0.000
n-3/n-6	0.73b	3.78a	0.26b	0.35	0.000
n-6/n-3	1.62b	0.36c	4.12a	0.33	0.000
PUFA/SFA	0.76	0.73	0.63	0.03	0.124
AI	7.97b	11.96a	5.79b	0.66	0.000
TI	1.02ab	0.79b	1.22a	0.05	0.000
h/H	2.28	2.08	2.16	0.05	0.272

C: control; FO: fish oil supplementation (3%); TMO: *T. molitor* larvae supplementation (10% for 3% oil); AI: atherogenicity index; TI: thrombogenicity index; h/H: hypocholesterolemic to hypercholesterolemic fatty acids ratio.

OP05 Possible Imported Soybean Alternatives for a Sustainable Broiler Production

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Abstract

The aim of this study was to determine the chemical compositions, amino acids, and fatty acid compositions of possible alternatives to soybean meal for sustainable broiler production. A total of 10 samples consisted of as follows: oil plant by-products (soybean meal, high protein sunflower seed meal, cotton seed meal, camelina meal), other plant by-products (dried tomato pulp, dried grape pomace (red wine), dried brewer's grain (barley)) and animal origin feed (de-mineralized whey powder, fish meal (*Engraulis encrasicolus)* and dried black soldier fly (BSF) larvae (*Hermetia illucens)* were studied. The dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, total sugar, calcium (Ca) and total phosphorus (P) were analyzed. Metabolizable energy (ME) was calculated from CP, EE, starch, and total sugar. Among the oil plant by-products, soybean meal had the highest CP, camelina meal had the highest EE, and sunflower seed meal had the highest CF. Dried brewers grain had higher CP, NDF, ME, Ca, total P, Σ PUFA, Σ omega-6 and Σ omega-3 values than the other plant by-products. The fish meal had the highest CA, CP, Ca, total P, lysine, methionine and Σ PUFA and Σ omega-3 values of all animal-origin feedstuffs studied. Dried BSF larvae had the highest ME value due to their highest EE value. The high prices of soybean have forced to search for alternative feed feedstuffs to soybean meal. Most of the feedstuffs presented in this study have the potential as an alternative to soybean.

Keywords: broiler, sustainability, soybean, alternatives, feed

Introduction

In Türkiye, a total of 2,297,071 tons of poultry meat was produced, including 2,245,770 tons of chicken meat and 58,301 tons of turkey meat in 2021. While the poultry meat industry is growing, issues mainly related to dietary protein sources are arising. It is crucial that the production of feedstuffs should be economical and sustainable. Chicken meat production currently relies on imported soybean. Soybean meal inclusion levels range from 25 to 40 % in chicken diets depending on the production type [1]. Soybean production already uses large areas of grassland which is associated with the conversion of forests into crop-production land, leading to biodiversity loss. In addition, the increase in soybean prices forces the poultry industry to find alternative protein sources [2].

At the farm level, formulation of broiler diets with the national and/or regional protein sources and by-products may reduce the use of soybean meal and contribute to the growth of the local economy. Possible alternative sources could be oil plant by-products such as sunflower meal, camelina oil meal, cottonseed meal, plant by-products such as dried tomato pulp, dried grape pomace, and dried brewers grain, and animal origin protein sources such as fish meal and whey powder. Black soldier fly (BSF) larvae, as an innovative alternative, are a high-value feed source; i.e. rich in protein and fat. Sunflower meal is considered a protein-rich but lysine-deficient and high-fibre ingredient [3]. Cotton seed meal can be used efficiently in broiler diets if enough digestible lysine is provided in the diet. However, its use is limited due to free gossypol presence [4]. Camelina oil meal has a high protein and oil content, but, due to its high fibre content and the presence of anti-nutritional factors and non-starch polysaccharides, it has low digestibility and a low metabolizable energy/gross energy ratio in poultry [5]. Camelina oil meal shows good potential for sustainable agriculture systems. It is often cited in organic agriculture literature because of its compatibility with minimum tillage systems [6]. Inclusion of plant by-products such as dried tomato pulp, grape pomace, and brewers grain in broiler diets is possible. Its low energy, high fiber value, and anti-nutritional factors in feed formulation should be taken into account. The production of plant by-products is seasonal and available only during the harvest period. Dried tomato pulp, grape pomace, and brewers grain can be an option for slow-growing broilers. However, the performance of these dried products is very limited for very young animals because of tannins [7]. The amount

of fat in BSF larvae is extremely variable and depends on the type of diet and on its fat content [8].

The present study aimed to determine the chemical compositions, amino acids, and fatty acids composition of possible alternatives to soybean for sustainable broiler nutrition.

Materials and Methods

The most promising soybean alternatives, oil plant by-products (soybean meal, high protein sunflower seed meal, cotton seed meal, camelina meal), other plant by-products (dried tomato pulp, dried grape pomace (red wine), dried brewer's grain (barley)) and animal origin alternatives (de-mineralized whey powder, fish meal (*Engraulis encrasicolus)* and dried black soldier fly (BSF) larvae (*Hermetia illucens)* were studied. Except for soybean meal all feedstuff samples were collected from the factory and the regional and or national feed producers. The dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, total sugar, calcium (Ca) and total phosphorus (P) were determined at Animal Science Department, Chemical Analyses Laboratory at Ege University, Faculty of Agriculture. The DM, CA, CP, EE, starch, total sugar, and Ca were determined based on AOAC [9]. CF was determined by Lepper [10]. NDF and ADF were determined based on Van Soest [11]. and total P was determined by spectrophotometer [12]. Metabolizable Energy (ME) was calculated as specified in TSE [13]. Amino acids and fatty acids were determined by HP at Central Analytical Laboratory (MATAL) at Ege University. The fatty acid compositions of the feedstuffs were determined based on Folch et al. [14] by gas-liquid chromatography (Waltham, MA, USA). Each analysis/ sample was replicated three times. The means were calculated and presented in Tables [15].

Results and Discussion

The chemical compositions, metabolizable energy (ME, kcal/kg on DM), some essential amino acids (% on CP), and total fatty acid composition (g/ 100 g lipid) were presented in Table 1. The detailed amino acid composition of feedstuffs (% on feed) to be used in broiler diet formulation was summarized in Table 2. The DM values ranged between 88.95 and 96.98 % (Table 1). Crude protein and EE levels of oil plant seeds measured in this study are in line with the ranges given in Feedipedia [16]. Among the oil plant by-products, the highest CP, EE, and CF were obtained in soybean meal, camelina meal, and sunflower seed meal, respectively, as expected. In addition, NDF was the highest in camelina meal, while ADF was the highest in sunflower seed meal. Starch was not detected by polarimetry at all oil plant by-products. The sugar was the highest in soybean meal. Camelina meal had the highest ME values compared to all oil plant by-products due to its high CP (although CP was 6.6 % lower than soybean meal), EE, and total sugar content. Cotton seed meal had the highest Ca and total P values. Lysine, methionine, and threonine are the highest in soybean meal, cotton seed meal and sunflower seed meal, respectively (Table 1). While the soybean meal had the highest Σ PUFA, the camelina meal had the highest Σ MUFA and Σ Omega 3. In addition, Σ SFA was the highest at sunflower seed meal, Σ omega 6 was the highest at cotton seed meal (Table 1). Results from this study suggested that cottonseed meal and camelina seed meal could be a good source of protein and energy for broilers when they were extracted by the expeller method. However, gossypol content makes cottonseed limited for young chicks [4]. In addition, considering fatty acid contents (Table 1), camelina seed meal can be a good source of fatty acids. However, antí-nutritional factors that lower the digestibility of camelina seed meal limit its inclusion in broiler diets [5]. High protein- low oil sunflower seed meal has a good potential for broiler diets but high CF (23.53 % DM) and low energy (2207 kcal/kg DM) as measured in our study and in Feedipedia [16] with a range between 16.1-37.4 % DM for CF and 2100 kcal/kg DM for ME limit the inclusion of young chick diets.

Among the other plant by-products, dried brewers grain had the highest CP, NDF, ME, Ca, Total P, Σ PUFA, Σ omega-6 and Σ omega-3 values. While dried tomato pulp had the highest CF, ADF, and methionine, dried grape pomace had the highest CA, EE, total sugar, lysine, threonine, Σ SFA, and Σ MUFA. The other plant by-products had higher CF, cell wall components, and Ca compared to soybean meal. On the other hand, essential amino acid values of all other plant by-products were higher than soybean meal except methionine of dried grape pomace and dried brewers grain (Table 1). Σ PUFA content of plant by-products was lower than soybean meal (Table 1).

When animal-origin feedstuffs were compared to soybean meal, the fish meal had the highest CA, CP, Ca, total P, lysine, methionine and Σ omega-3 values, as expected. Therefore, the fish meal has a high biological value not only as a protein and lysine source but also as a source of minerals [16]. However, the high price and lack of standardization limit its usage. Whey powder had higher starch and Σ MUFA than soybean meal (Table 1). Because of the highest EE values of the dried BSF larvae, ME values were higher than all feedstuffs. Additionally, the analytical CF of the dried BSF larvae was higher than the soybean meal. Dried BSF larvae had the highest threonine and Σ SFA compared to the all feedstuffs studied (Table 1). Considering the organic waste consumption capacity and the nutrient composition of BSF larvae (Tables 1 and 2), BSF

larvae can be accepted as an environmentally sustainable alternative feedstuff. European Commission approved BSF larvae usage in 2021 (COMMISSION REGULATION (EU) 2021/1372 of 17 August 2021) in pig and poultry diets. However, the commercialization of BSF larvae production is limited in the EU, Canada, and the USA [17]. It is expected that BSF larva production will increase in the future in our country after following a similar regulation to the EU.

Conclusion

The high prices of soybean have forced to search for alternative feedstuffs to soybean meal. Most of the feedstuffs presented in this study have the potential as an alternative to soybean. However, the use of alternative feedstuffs depends on not only nutritional composition, digestibility, and anti-nutritional factors but also on price and availability. On the other hand, it is important and possible to improve the quality of these alternative protein sources through processing or supplementation with nutrient and non-nutrient additives such as enzymes. With the increase in soybean prices, studies on searching possibilities of using alternative feedstuffs will continue.

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Table 1. The chemical compositions (% on DM), metabolizable energy (ME, kcal/kg on DM) values, essential amino acids (% on CP) and total fatty acid compositions (g/ 100 g lipid) of feedstuffs

	OIL PLANT BY-PRODUCTS				OTHER PLANT BY-PRODUCTS			ANIMAL-ORIJIN FEEDSTUFFS*			SEM
	Soybean meal	Sunflower seed meal (high protein)	Cottonseed meal	Camelina meal	Dried tomato pulp	Dried grape pomace	Dried brewers grain	Whey powder (de-mineralized)	Fish meal	Dried BSF Larvae	
Chemical Composition											
Dry matter, %	88.96	88.95	89.36	93.10	93.62	92.70	96.98	94.86	92.29	95.53	0.61
Crude ash	7.29	6.66	9.11	5.24	7.42	15.48	4.16	5.58	12.07	5.51	0.76
Crude protein	49.26	43.51	38.28	43.50	16.14	23.20	23.86	8.85	65.17	38.32	3.68
Ether extract	0.98	1.95	4.72	5.77	1.39	3.25	2.50	0.39	10.75	34.51	2.23
Crude fiber	7.42	23.53	11.44	11.23	45.51	17.46	18.86	10.37	0.35	11.77	2.66
NDF	20.57	34.90	26.96	36.03	59.88	49.32	70.24	-	-	16.77	5.06
ADF	11.09	30.73	15.62	14.73	46.86	27.74	27.82	-	-	12.76	3.19
Starch	-	-	-	-	-	-	2.80	20.06	-	-	1.37
Total Sugar	9.70	8.20	6.94	9.58	2.32	2.90	1.92	-	-	1.79	0.84
ME, kcal/kg DM	2403	2207	2195	2586	881	1332	1369	1264	3454	4534	243
Ca, g/kg DM	2.17	4.38	12.88	3.87	18.17	12.52	23.77	6.83	17.42	16.94	2.31
Total P, g/kg DM	7.22	9.67	11.34	7.53	3.94	6.30	7.36	5.98	23.81	5.65	1.78
Essential Amino Acids											
Lysine	3.26	3.12	2.89	2.54	4.63	6.49	3.70	4.05	6.14	3.52	0.30
Methionine	2.68	0.62	3.15	0	4.00	0.84	0.93	1.13	3.23	1.68	0.29
Threonine	3.06	4.57	3.37	3.30	4.14	6.81	4.56	3.04	0	7.16	0.44
Total Fatty Acids											
Σ SFA	22.530	30.794	27.301	13.035	21.923	43.745	28.097	58.891	37.827	72.030	3.947
Σ MUFA	20.947	28.760	18.022	44.359	26.826	27.093	18.438	34.972	30.495	16.962	1.883
Σ PUFA	56.508	40.446	54.777	42.411	51.220	29.162	53.465	6.138	31.250	11.083	3.927
Σ Omega 6 (n-6)	49.799	37.469	53.903	24.971	49.804	15.201	51.271	6.138	6.142	9.708	4.390
Σ Omega 3 (n-3)	6.245	1.996	0.388	13.793	0.820	-	1.406	-	15.493	0.930	1.277
n-6/n-3	7.974	18.772	139.083	1.810	60.716	-	36.463	-	0.396	10.442	-

BSF, black soldier fly; **DM, dry matter**; ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber ; Ca, calcium, P, phosphorus; Σ SFA, total saturated fatty acids; Σ MUFA, total monounsaturated fatty acids; Σ PUFA, total polyunsaturated fatty acids, SEM, Standard error of means, Values not detected (-)

*Crude fiber, NDF and ADF values were determined analytically.

Table 2. Amino acid compositions of the feedstuffs (% on feed)

	Oil Plant By-Products				Other Plant By-Products			Animal Orijin Feedstuffs			
	Soybean meal	Sunflower seed meal (high protein)	Cottonseed meal	Camelina meal	Dried tomato pulp	Dried grape pomace	Dried brewers grain	Whey powder (de-mineralized)	BSF Larvae (Dried)	Fish meal	SEM
Aspartic Acid	3.060	2.855	2.415	2.045	0.690	1.820	1.085	0.450	2.160	3.230	0.212
Glutamic Acid	3.670	4.985	3.930	3.465	1.030	1.695	2.780	0.645	1.750	3.815	0.311
Asparagine	-	-	-	0.010	-	-	-	-	-	-	0.0006
Serine	1.155	1.205	1.040	0.985	0.315	0.830	0.690	0.205	0.800	-	0.090
Glutamine	0.020	0.095	0.030	0.020	-	0.020	0.040	-	-	-	0.006
Histidine	0.300	0.355	0.310	0.255	0.095	0.250	0.205	0.040	0.335	-	0.028
Glycine	1.710	3.245	1.685	2.025	1.175	1.855	1.350	-	-	-	0.230
Threonine	1.340	1.770	1.155	1.335	0.625	1.465	1.055	0.255	2.620	-	0.164
Arginine	2.250	2.885	2.350	2.745	1.085	1.220	2.330	2.605	6.345	-	0.362
Alanine	1.925	1.565	2.270	2.125	1.210	1.865	1.055	0.265	1.820	-	0.168
Tyrosine	0.485	0.445	0.420	0.350	0.380	0.360	0.380	0.055	0.730	1.915	0.110
Cystine	0.050	0.095	0.060	0.070	0.045	0.055	0.050	0.010	0.050	0.125	0.007
Valine	0.215	2.005	0.260	1.570	0.215	1.520	1.430	0.330	1.520	4.965	0.311
Methionine	1.175	0.240	1.080	-	0.605	0.180	0.215	0.095	0.615	1.945	0.135
Norvaline	0.115	0.280	0.120	0.145	0.195	0.140	0.150	-	-	-	0.020
Tryptophan	0.010	0.010	0.010	0.275	0.010	0.010	0.005	0.025	0.050	0.215	0.021
Phenylalanine	1.175	1.440	0.805	0.970	0.540	1.070	0.995	0.280	0.625	2.585	0.138
Isoleucine	0.960	2.820	1.485	-	0.580	0.670	0.855	0.110	0.850	3.575	0.251
Leucine	2.090	2.320	1.610	1.755	0.820	1.620	1.075	0.485	1.500	3.560	0.188
Lysine	1.430	1.205	0.990	1.030	0.700	1.395	0.855	0.340	1.290	3.690	0.197
Hdroxyproline	1.095	0.965	0.795	0.885	0.715	1.210	0.765	0.325	0.825	4.175	0.235
Sarcosine	0.095	0.770	0.135	0.455	0.280	0.845	0.095	-	0.090	0.570	0.067
Proline	0.030	0.695	0.020	0.205	0.040	0.605	0.035	0.025	0.525	0.215	0.058

SEM, Standard error of means, Values not detected (-)

IS[04] Novel Approaches to Control Avian Influenza and Newcastle Disease in Poultry

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Poultry production is characterized by a huge diversity of farming systems, with different scales of production, bird species, measures of biosecurity, production inputs and outputs. Both intensive commercial poultry farms and backyard flocks coexist, with very different characteristics. However, both commercial and backyard farming systems use animals that are susceptible to the same diseases. This might represent a high risk for the transmission of infectious agents between these two compartments. Furthermore, the importance of biosecurity measures for large-scale poultry production is well understood. However, its significance for backyard flocks should not be underestimated since they could a source of infection for the commercial poultry (Steenwinkel et al., 2011).

Avian Influenza (AI) and Newcastle disease (ND) are the most important threats to systematic poultry farming, as well as a significant risk to public health. Their importance lies in the fact that they affect birds health, public health and trade both domestically and internationally. Mortality can even reach 100%. They are among the most contagious viral diseases of birds, although incidents have also been reported in mammals, such as humans, dogs, cats, pigs, horses, etc. In the event of an outbreak, to prevent their spread, exports are prohibited and two zones of 3 and 10 kilometers (Protection Zone and Surveillance Zone) are created around the outbreak, in which the movement of live birds is prohibited. The above measures have a heavy impact on the poultry businesses, while in case that the production units of the businesses (hatchery, abattoir, meat factory, egg candling, etc.) are located within the above zones, it could lead to incalculable damages for the poultry businesses and subsequently for the local economy-society (Swayne et al., 2020).

Some strains of the High Pathogenic Avian Influenza (HPAI) virus can mutate and pose a serious threat to humans, as happened in 2005 with the H5N1 strain and as is now the case with the H7N9 strain in SE Asia. Several AI and ND epidemics have been reported in the past, with incalculable losses in livestock, human life and money. The recent avian influenza epizootic in Europe in 2021–2022 is the largest AI epizootic observed so far in Europe, with a total of 2,467 cases resulting in the death of 47.7 million birds. The HPAI virus has been detected in wild birds with an unprecedented geographical spread reaching from the Svalbard Islands to southern Portugal and Ukraine, affecting 37 European countries. In Greece, HPAI was detected in 2016-2017 for first the time in a commercial layer farm, while there were also 13 incidents in wild and backyards birds (EFSA et al., 2022).

The diseases are seasonal, with the highest incidence occurring in autumn. Aquatic and wild birds are the reservoir of HPAI and ND viruses in nature. A dynamic cycle of infection occurs within aquatic and wild avian species, many of which are migratory, as well as between these birds and commercial poultry and other animals. Spillover of viruses from wild aquatic birds to poultry and other species occurs frequently. HPAI and ND viruses can spread globally through migratory birds and cause massive outbreaks in commercial poultry. Outbreaks have been associated with proximity to waterbodies, presence of waterfowl or wild bird cases near poultry farms (Velkers et al., 2020). Contact with infected wild and aquatic birds is their gateway to countries and backyard poultry flocks with a direct risk of transmission to systematic farms. In addition,

the knowledge of environmental and ecological drivers for aquatic and wild bird presence and abundance as well as back yard poultry flocks location could be very helpful to identify and select the high risk areas for surveillance and biosecurity measures. However, the demographic structure and dynamics of small-scale poultry farms is challenging as a result of the lack of systematic accounting of birds' entries and exits on farms, the absence of all-in-all-out management, the simultaneous presence of birds of different species and production stages on the same farm, and the combination of self-renewal and outsourcing of young birds from other farms and/or hatcheries (Delabouglise et al., 2019; Velkers et al., 2020).

The probability of HPAI and ND viruses introduction in a country or area and spread is determined by a complex combination of factors, such as the number and density of commercial and backyard poultry farms, the type of species or breeds present, the number and type of birds of the farms, and the sanitary measures that are put in place. To avoid the introduction of diseases into farms and to contain the spread of infections already present, appropriate preventive measures need to be applied. The implementation of strict biosecurity measures to avoid contact between backyard and commercial poultry flocks with wild birds, as well as the immunosurveillance of wild and aquatic birds is the cornerstone for reducing the risk of outbreaks of AI and ND, as well as to prevent the AI virus from mutating into a pathogenic strain, with incalculable consequences for both birds and humans (Steenwinkel et al., 2011).

The risk of ND and especially AI outbreaks is continuous and uninterrupted, making the surveillance of backyard poultry flocks an essential tool for the early and timely diagnosis of diseases. The installation of a prototype network for the early detection of HPAI and ND viruses will significantly contribute to the early diagnosis of the above diseases, and will give the necessary time to the veterinary service and the poultry farms of the continent region to take the necessary measures to contain and preventing their spread in systematic poultry farms, as well as in humans. Especially in the case of AI, the systematic observance of biosecurity measures is crucial, given the speed of transmission of the virus, the peculiarities of its epidemiology and the magnitude of the economic impact caused by its possible uncontrolled spread (Rodríguez-Prieto et al., 2015).

The EPIRORNIS is a research program for the early diagnosis of the above diseases, which contributes significantly to the prevention of AI and ND in the Region of Epirus, which is characterized by a high concentration of poultry businesses. In particular, the program includes the sampling of sentinel birds in high-risk areas (near reservoirs, in breeding hens and egg-producing hens) as well as in areas where vital production units of the cooperating companies are located. The sentinel birds come from the existing backyard poultry flocks located in the specific areas, after recording them and tagging the birds. A clinical examination of the birds is carried out daily, while in case of mortality a necropsy is carried out. Sampling is done monthly, while their frequency is doubled during the high-risk period (October-April). Sampling includes both serological (ELISA) and molecular tests (PCR) to detect antibodies and virus, respectively. Corresponding tests are also carried out in flocks of intensive and organic rearing of meat-producing and egg-producing hens.

The implementation is considered necessary for Greece and in particular for the region of Epirus, where is located more than 50% of Greek poultry industry, contributing to the increase and development of local economy and society. The operating a surveillance system for the detection of the virus in wild birds and backyard poultry flocks and the development of a preparedness plan for the prevention of systematic poultry breeding units is a great advantage for the wider region of Epirus where systematic poultry farming, the most dynamic branch of Greek livestock, marked a leap forward in terms of production, structure and financial result. By utilizing the results of EPIRORNIS we can improve the health conditions of the region's livestock, laying a solid foundation for the further development of systematic poultry farming, strengthening the growth and competitiveness of businesses through the integration and utilization of new knowledge in it, with profit the multiplier benefits that flow from it. Contributing to the prevention of AI and ND, which are the most important threats to the viability and productivity of systematic poultry farming, will protect poultry businesses and enable them to maintain the steady upward trend they have shown in recent years.

Keywords: Biosecurity, campylobacteriosis, avian influenza, Newcastle disease, poultry, control

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OP06 Algal Beta-(1,3)-Glucan and Its Effect on Infectious Bursal Disease Vaccination

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Abstract

Vaccination is used as a control and prevention tool for Infectious bursal disease (IBDV). A new strategy in improving vaccination efficiency is the use of in-feed immune modulating ingredients. The aim of this trial was to test if the use of beta-(1,3)-glucans in feed could enhance IBDV vaccination. A trial was conducted in ROSS 308 broilers. The treatments consisted of a negative control group (not vaccinated, not supplemented), a positive control group (vaccinated, not supplemented) and a treated group (vaccinated, supplemented with beta-(1,3)-glucan). All broilers, except the negative control, were orally vaccinated at 18 days of age with a live IBDV vaccine. Blood samples were taken at day 18 and 35 to measure antibody titers against IBDV. Serological analysis showed the presence of maternal derived antibodies at time of vaccination in some birds. The beta-(1,3)-glucan supplemented birds showed decreased CV% (coefficient of variation) and significantly increased average antibody titers compared to non-supplemented vaccinated birds. Additionally, beta-(1,3)-glucan group showed increased number of birds with antibody titers above the titer threshold for protective immunity. The results obtained in the current trial clearly indicate that beta-(1,3)-glucan can increase seroconversion and serological response to IBDV vaccination.

Keywords: beta-(1,3)-glucan, *Euglena gracilis,* broilers, Infectious Bursal Disease, vaccination

Introduction

Vaccination plays a vital role in poultry health management. Immunization has known a growing interest since the ban on the use of antibiotic growth promotors in animal production and is an important tool to reduce the use of therapeutic antibiotics. The primary reason for vaccinating poultry is to reduce the losses due to morbidity and mortality caused by all kind of pathogens (Marangon and Busani, 2007). There are plenty of diseases that are prevented by vaccinating animals against them. One of the most common viral infections in chickens, infectious bursal disease (IBDV), also known as Gumboro disease, is caused by the IBDV virus. This virus destroys B-lymphocytes in the bursa of Fabricius and affects cell-mediated immunity, leading to mortality and immunosuppression resulting in poor performance with significant economic impact (Sharma *et al*., 2000; Müller *et al*., 2012; Ingrao *et al.*, 2013). Vaccination is most important in aiding in IBDV prevention and control (Marangon and Busani, 2007; Müller *et al*., 2012). The vaccine helps to prevent IBDV by inducing a cell-mediated immunity and by boosting the animals' humoral immune system to produce antibodies that in turn fight the invading virus, protecting them against IBDV (Jakka *et al.*, 2014). There are a lot of factors determining vaccination efficiency. A vaccination failure is defined as: "when the animals do not develop adequate antibody titer levels and/or are susceptible to a field disease outbreak, following vaccine administration" (Sharif and Ahmad, 2018). Often the vaccine is blamed, but a lot can go wrong in between vaccine development and preparation, and the production of neutralizing antibodies by the animal (Marangon and Busani, 2007; Sharif and Ahmad, 2018). Monitoring antibody titers is important to guarantee efficient vaccination and to allow adjustment of vaccination programs, e.g. in case of presence of protective IBDV maternal antibodies acquired through the egg yolk, the IBDV live vaccine will be neutralized, consequently resulting in vaccine failure (Marangon and Busani, 2007; Müller *et al*., 2012). A tool in improving vaccination efficiency not systematically used yet, is the use of an in-feed supplementation modulating the immune system. Research has shown response to vaccination can be improved by using immune modulating ingredients administered through the feed, such as beta-glucans (An *et al.*, 2008; Kovacocyova *et al*., 2014; Vojtek *et al*., 2017; Horst *et al.*, 2018). Antigen-presenting cells (such as macrophages and dendritic cells) can recognize beta-(1,3)-glucan carbohydrate structures by specific receptors on their surface (such as the dectin-1 receptor) (Medzhitov, 2007; Goodridge *et al.*, 2009; Soltanian *et al*., 2009). In response to binding beta-(1,3)-glucan, those immune cells will become more active in engulfing, killing and digesting invading pathogens and will initiate a signaling cascade stimulating the attraction, formation and activation of other immune cells (Soltanian *et al.*, 2009).

One organism effectively producing beta-(1,3)-glucans, is the alga, *Euglena gracilis*, as this organism stores the molecule as a carbohydrate product in its cytoplasm (Krajcovic et al., 2015). This new algal-derived beta-(1,3)-glucan is available as an

in-feed solution for all animal species. The aim of this trial was to test if the use of this new algal- derived beta-(1,3)-glucan in feed could enhance IBDV vaccination efficiency in poultry.

Materials and Methods

Birds and management :The broiler trial was carried out during six weeks in a semi-commercial facility in Belgium. In total 96, individually tagged one-day-old male Ross 308 broilers were divided into 3 treatment groups. The 32 birds (replicates) per treatment group were housed in 8 separate pens (4 birds/pen). The pens were littered with wood shavings and birds were fed using a 2-phase feeding scheme: starter (d 1 to 14), grower-finisher (d 14 to 38). Drinking water and feed (mash form) were provided ad libitum. The composition of the basal diet can be found in Table 1. Treatments were assigned to pens using a randomized complete block design. The treatments consisted of a negative control group (no vaccination, basal diet), a positive control group (vaccination, basal diet) and a treatment group (vaccination, basal diet supplemented with 50 g/T beta-(1,3)-glucans from *Euglena gracilis, (*Aleta™, Kemin Europa NV), throughout the whole feeding period. The negative control group was housed in a designated isolated area and a strict protocol (specific sequence, protective clothing) was followed when entering the broiler house. On day 18 (Fantay *et al.*, 2015), the broilers in the vaccinated groups were individually vaccinated with an oral live freeze-dried IBDV vaccine (Nobilis® Gumboro D78, MSD Animal health).

Blood sampling : On d18 and 35, blood samples were taken from the wing vein (*vena cutanea ulnaris*) from all animals. Serum was separated by centrifugation at 3000 g for 10 minutes and the serum antibody titers (IgY) against IBDV were measured using a commercial IBDV ELISA kit (Biochek, United Kingdom) following the instructions of the manufacturer. Measuring antibody titers on day 18 is important to detect if maternal antibodies, which can interfere with the vaccine, are still present, consequently making the bird less susceptible for the vaccination. Analysis of variance (ANOVA) was applied for statistical analysis (Statgraphics Centurion XVI software, Statpoint Technologies, Warrenton, VA, USA). Means were compared using Fisher's least significant difference procedure. All statements of significance were based on a *P*-value less than or equal to 0.05.

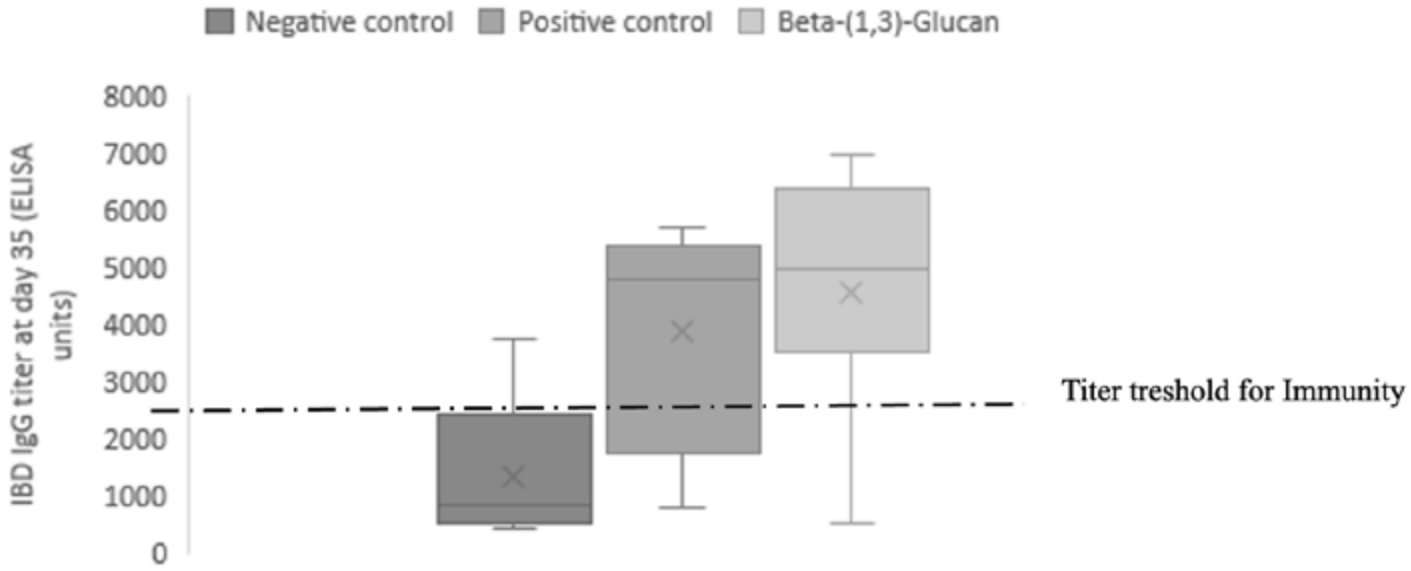
Results and Discussion

Because of the endemic situation it is advised to vaccinate birds against IBDV in Belgium (DGZ, 2018). Vaccination is performed by a live vaccine via drinking water or by a recombinant vaccine via subcutaneous injection. Laying and breeder hens are vaccinated at day 1, day 20 or day 28, depending on the presence of passive immunity acquired through the yolk. Broilers are vaccinated in between day 10 and 18, depending on their immune status (WVPA, 2015). To guarantee protection against IBDV it is important to determine the optimal vaccination age. Optimal time of vaccination is routinely determined by serological examination of serum (ELISA) in one- to three-day old broilers using the Deventer formula (Smialek *et al*., 2016). If serology shows the presence of maternal antibodies, vaccination should be postponed to a later age, as those antibodies will neutralize the vaccine (Marangon and Busani, 2007; Müller *et al.*, 2012). Still, routine serology is often neglected, and vaccination is performed at standard ages (DGZ, 2016). It is clear the standard advised vaccination program regarding IBDV is not easy applicable in the field.

The trial described in the current paper clearly shows the presence of maternal antibodies (in about 30% of the birds), as already documented by several authors (Naqi *et al.,* 1983; Alam *et al.*, 2002; Müller *et al*., 2012) in case of IBDV. Serological analysis at day 18 showed the presence of maternal IBDV specific antibodies (MDA) in 22 out of 96 birds with an average antibody titer of 530 ELISA Units (Table 2). No statistical difference was observed in average MDA titers between groups. A clear presence of passive immunity could interfere with IBDV vaccination programs and therefore serology should be performed on a routine base to determine the optimal time of vaccination. As the optimal antibody titer to vaccinate should be equal or below 100-250 ELISA units (Biochek, 2017) when using an intermediate live vaccine such as Nobilis Gumboro D78, birds showing passive protection at day 18 were taken out of the trial.

At 35 days, differences in seroconversion to the vaccines were observed amongst all treatment groups (Figure 1). In total, 52% of the birds reacted positively to the vaccine in the beta-(1,3)-glucan supplemented group, while only 25% and 24% of the birds seroconverted in the positive and negative control group respectively. Results of the average IBDV titers are shown in Table 3. Nobilis Gumboro D78 vaccines should induce immunity 6-7 days after vaccination and protective immunity should remain 31 days as claimed by the vaccine producer. This was clearly shown by the serological analysis on day 35 (17 days after vaccination). The non-vaccinated birds showed a non-protective average IgY titer compared to both vaccinated groups with protective average titers. Furthermore, the average IBDV titer at day 35 was significantly increased in the beta-(1,3)-glucan supplemented group (4563.3 ELISA units) compared to the positive (3905.8 ELISA units) and negative (1348.7 ELISA units) control group (Table 3).

To evaluate the success of vaccination, two parameters need to be considered: the mean antibody titers (as a measure of the immune response to the vaccine) and the coefficient of variation (CV%) (showing the uniformity of the vaccination response



in a group or flock). For live vaccine applications, the CV% should be below 60% for effective and homogenous vaccination (Biochek, 2017), which was the case in both vaccination groups (50.80% and 47.46%, Table 3). A numerical decrease of the CV% was observed in the beta-(1,3)-glucan supplemented group (47.46%), compared to the positive control group (50.80%). The CV% was above 60% in the non-vaccinated group, confirming the hypothesis that the positive antibody titers in this group were generated by circulation of the vaccine virus in the house and not by active immunization.

The main antibody titers at day 35 in broilers vaccinated with Nobilis Gumboro D78 should be above 2500 ELISA Units to guarantee protection (Biochek, 2017). Considering the antibody titers in the vaccination groups were above the protective threshold and had a CV% below 60%, the vaccination performed in the current trial can be considered as successful. Additionally, the broilers in the beta-(1,3)-glucan supplemented group showed significantly higher antibody titers (4563.3 ELISA units) and lower CV (47.5 %) compared to the non-supplemented vaccinated control group, providing a significantly better vaccination efficiency.

When the antibody titers observed in the different treatments were plotted and compared to the threshold for immunity of 2500 ELISA units in case of Nobilis Gumboro D78 vaccination in broilers (Figure 2), the following conclusions could be made. In the negative control group, 20% of birds reacting positively to the vaccine showed protective immunity, hypothetically due to circulation of the vaccine strain in the broiler house as noted before, although protective measures to prevent circulation of the virus in the poultry house were taken, such as a designated isolated area for the negative control birds, strict treatment sequence, protective clothing. Further virological analysis to confirm this hypothesis was not performed. In the vaccinated groups, 66.67% and 84.62% of birds seroconverting to the vaccine (which were 25% and 52%) showed titers above the protective immunity threshold, in positive control and beta-(1,3)-glucan supplemented group respectively. Beta-(1,3)-glucan supplementation increased the number of birds showing protective immunity compared to the non-supplemented birds, as can be observed in Figures 3 and 4. This effect has been shown previously by Horst *et al.* (2018), who investigated the effect of beta-(1,3)-glucan supplementation on NDV vaccination. In that study, beta-(1,3)-glucan supplementation of birds resulted in increased number of birds with NDV antibody titers above the immunity threshold compared to the non-supplemented group.

Conclusion

The results of the trial in the present paper show that beta-(1,3)-glucan can increase IBDV seroconversion and serological response. Beta-(1,3)-glucan supplemented birds showed increased average antibody titers and decreased CV% compared to non-supplemented vaccinated birds. Additionally, the beta-(1,3)-glucan supplemented group showed increased number of birds with antibody titers above the titer threshold for protective immunity. These data prove that beta-(1,3)-glucan supplementation can increase the success rate for an efficient vaccination.

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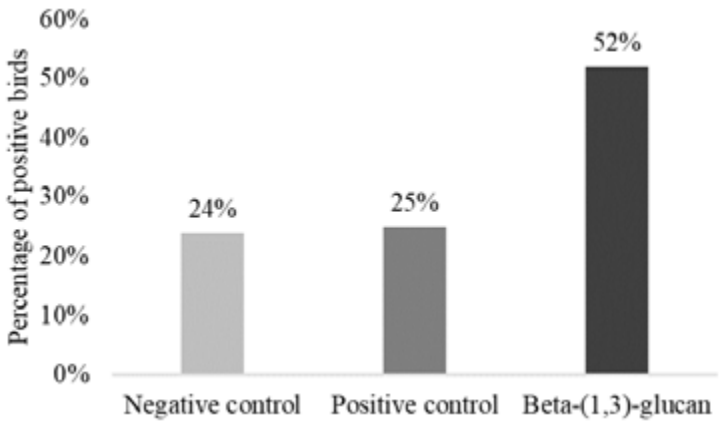

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Table 1. Composition of the starter and grower basal diet.

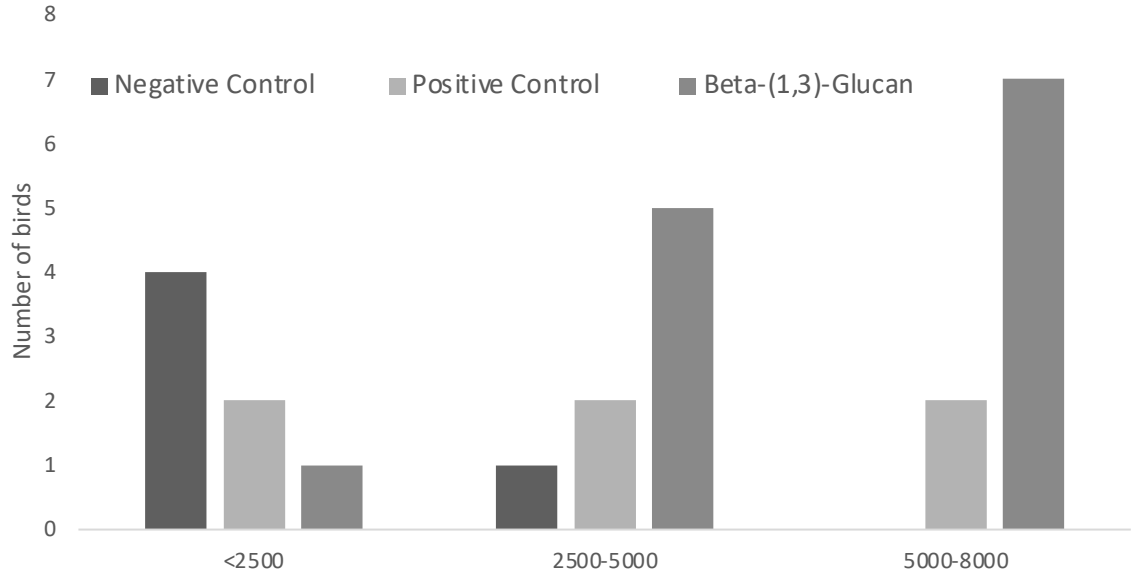
Ingredients (%)	Starter (0-14d)	Grower (15-35d)
Corn	40.00	10.00
Soybean meal	22.45	12.60
Wheat	16.99	50.40
Soy	15.00	18.59
Vitamin/mineral premix	1.33	1.33
Limestone	1.09	1.39
L-lysine hydrochloride	1.09	1.02
Animal Fat	1.00	3.00
Monocalcium phosphate	0.56	0.33
DL-Methionine	0.28	0.24
Soya oil	0.10	0.00
L-Threonine	0.07	0.00
Sodium chloride	0.03	0.02

Figure 1. Percentage of seroconverted birds (positive birds) at day 35 (average of 32 replicate birds per group).

Table 2. Average specific IBDV MDA (infectious bursal disease virus maternal antibodies; ELISA units) at day 18 and IgY titer (ELISA units) at day 35 (the values are shown as means of 32 replicate birds ± standard deviation).

	MDA (ELISA units)	IgY (ELISA units)	CV %
Negative Control	531.5 ± 192.5	1348.7 ± 1361.7a	101.0
Positive control	513.8 ± 137.6	3905.8± 1984.5a	50.8
Beta-(1.3)-glucan	548.4 ± 174.2	4563.3 ± 2165.9b	47.5
	P-value = 0.402	P-value= 0.007	

Figure 2. Box plot of IBDV titers (Infectious bursal disease virus; ELISA units) per treatment at day 35 (the boxes show the first quartile, median and third quartile; the whiskers show the minimum and maximum; the x shows the mean value).



OP07 Monitoring the Effectiveness of Immune Complex Vaccine Against IBD in Broilers

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Abstract

Infectious bursal disease, also known as Gumboro disease, is an economically important viral disease that causes immunosuppression and lesions in the bursa Fabricius in broilers, layers, and breeders. Vaccination is often the preferred method of controlling the disease. The aim of this study is to compare the effect of antibody levels at different weeks on the serological response of broiler flocks with different maternal antibody levels vaccinated with immune complex vaccine against infectious bursal disease and to detect the vaccine virus in bursa Fabricius and spleen samples by quantitative RT-PCR. Specific antibodies were determined by ELISA test by taking 23 blood serums from 5 broiler flocks with different maternal antibody titers on day 0, day 21 and just before slaughter. The presence of bursa Fabricius and spleen vaccine strains taken separately from 5 animals on the 28th day of production were comparatively and quantitatively examined by real-time PCR (qRT-PCR). It was determined that maternal antibody level among herds varied between 5645 and 8075. It was noted that the specific antibodies ranged between 95 and 1003 on the 21st day. It was observed that the specific antibodies in the blood serum taken just before slaughter varied between 4091 and 6950. It was found that 5 bursa Fabricius and spleen samples taken individually on the 28th day of the vaccine strain increased at rates ranging from 90% to 100%. According to the findings obtained, it was determined that specific antibodies started to form after the 21st day following the immune complex vaccination of herds with different maternal antibody titers and increased until slaughter. It was noted that herds with high maternal antibody titer had lower specific antibodies at slaughter stage. Real time-PCR (qRT-PCR) was found to be very useful in the follow-up of vaccination success.

Keywords: Broiler, immun complex vaccine, infectious bursal disease, vaccination efficiency

OP08 Current status of IB Virus Israel Variant-2 (GI-23) Genotype in Turkiye and Phylogenetic Analysis Based on S1 Gene

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Abstract

Infectious bronchitis (IB) is an important disease that causes severe economic loses in the poultry industry worldwide. Furthermore, the spread of new variants poses a challenge for diagnosis and control of the disease. This study investigated the situation of infectious bronchitis virus (IBV), specifically the Israel variant-2 (IS var-2) also known as GI-23 genotype, in Turkey. Between 2014 and 2019, 214 flocks vaccinated against H120 from Marmara, Western Black Sea, and Inner Anatolia were examined, with 127 (59.3%) flocks testing positive for IBV, of which 92 (72.4%) were positive for IS var-2. Of the latter samples, 60 were randomly selected and subjected to full S1 gene sequencing. The analysis indicated that the field strain in Turkey was located on the same branch as the GI-23 genotype, which is one of the most frequently observed genotype found in the Middle East. The DNA similarities between the GI-23 isolates from 2014 to 2019 were 99%. In conclusion, the IS var-2 genotype has been circulating in broiler flocks in Turkey.

IS[05] Alternative Hatching Systems in Broiler Chickens

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Summary

In the current conventional hatching system, environmental conditions, such as temperature, level of dust, use of disinfectants may be suboptimal for chickens and additionally, chickens lack access to feed and water until placement in the rearing house, which might be suboptimal as well. These both suboptimal aspects have led to development of alternative hatching systems. These alternative hatching systems differ in various ways from the conventional hatching system and these differences may affect day-old chicken quality as well as later life health, welfare and performance. In this conference paper, alternative hatching systems will be compared with the conventional hatching system. First, differences in environmental and management factors will be discussed, followed by the consequences of alternative hatching systems for day-old chicken quality, health, welfare and production. It can be concluded that alternative hatching systems might be less challenging for chickens than the conventional hatching system, but effects on day-old chicken quality, and later life health, welfare and performance are ambiguous. For optimal chicken health, welfare and performance, settings in both the conventional and alternative hatching systems are more important than the hatching system itself.

Introduction

The day-old broiler chicken is a crucial hinge between the breeder and the broiler (Decuypere et al., 2001) and quality of the day-old chicken appears to be related to later life health, welfare and performance (Tona et al., 2005; Willemsen et al., 2008; van der Wagt et al., 2020). In the current conventional hatching system, two important aspects potentially reduce the quality of day-old chickens:1) Suboptimal environmental conditions in the hatcher and 2) The length of the hatch window in relation to first access to feed and water. Firstly, environmental conditions in the hatcher might be suboptimal regarding high temperature, high dust levels and use of disinfectants, which might negatively affect day-old chicken quality (Zulkifli et al., 1999; Leksrisompong et al., 2007; De Gouw et al., 2017). Secondly, chickens hatch over a hatch window of approximately 24 to 36 hours (Careghi et al., 2005; Willemsen et al., 2010) and are removed from the hatchers only when the majority of chickens has hatched. Chickens that hatch particularly early during the hatch window face a long period without feed and water before they are removed from the hatcher and placed in the broiler house. These two aspects may impair health and welfare of chickens at both hatch and in later life. This has led to the development of alternative hatching systems, which differ on several aspects from the conventional hatching system. The aim of the review paper is to discuss differences between the conventional and alternative hatching systems and to investigate effects of different hatching systems on day-old chicken quality as well as on health, welfare and performance in later life.

Differences between the conventional and alternative hatching systems

Current alternative hatching systems can be divided into two categories: 1)Chickens hatch in a hatchery and have feed (and water) available in the hatcher (hatchery-fed). Examples of these systems are SmartStart or HatchCare; or 2) On-farm hatching systems in which, after candling at day 18 of incubation, fertile eggs are transported to the rearing farm, where they hatch. Examples of on-farm hatching systems are Patio, X-treck, One2Born or NestBorn.

An important difference between conventional and alternative hatching systems is the immediate access after hatching to feed (and water) in alternative systems, whereas this is delayed in the conventional hatching system. However, there are several other differences between both systems, which can be divided into environmental and management factors. Environmental factors are:

- Climate. Most important aspects related to climate are the temperature (Lourens et al., 2005), air velocity (Meijerhof and Van Beek, 1993), relative humidity (Bruzual, 2000) and carbon dioxide concentration (Van den Brand et al., 2021). All these climate factors are important to create optimal conditions during the hatching phase. Alternative hatching systems

differ in all these aspects from the conventional hatching system, but in both types of systems, optimal climate settings are possible.

- Egg position. In alternative hatching systems, eggs are positioned in a vertical (blunt end up) or horizontal position. Hardly any work has been done on effects of egg position on chicken quality. Van de Ven et al. (2011) did not find differences in chicken quality or hatchability when eggs were kept in a horizontal, vertical blunt end up or vertical blunt end down position.
- Light. In the conventional hatching system, normally no light is provided, but in alternative hatching systems light is in most cases provided. Light can penetrate the eggshell of broiler eggs (Güz et al., 2021) and reach the embryo. It can be questioned whether or not exposure of eggs to light for only 3 to 4 days during the hatching phase will have large effects on hatchability and day-old chicken quality.
- Noise. Forced-ventilated hatchers make a lot of noise (often >85dB), meaning that they might disturb vocal communication among embryos and may consequently affect hatchability, hatching time and hatching synchronisation. It is possible that machine noise in conventional hatchers masks the potential benefits of species-specific vocalisations, as suggested by Donofre et al. (2020). Consequently, it can be speculated that on-farm hatching with lower noise levels might reduce the hatch window, as suggested by Van de Ven et al. (2010), though this has not been extensively investigated.
- Dust and use of disinfectants. In conventional hatching system, day-old chickens are commonly exposed to high levels of dust (Mitchell and Waltmann, 2003), high pathogen loads (Mitchell and Waltmann, 2003) and disinfectants (quite often formaldehyde) (Zulkifli et al., 1999). Disinfectants cannot be applied in all alternative hatching systems. In on-farm hatching systems, the air volume per egg is considerably higher than in the other systems, reducing the concentration of dust and pathogens on chicken level. Consequently, the use of disinfectants during the hatching phase is likely to be of less importance. However, the use or lack of use of disinfectants in alternative hatching systems is hardly investigated.

Management differences among hatching systems are the handling and transportation of chickens, the removal of second-grade chickens and the immediately access to feed and water after hatching.

- Chicken handling and transportation. In the conventional hatching system, chickens are removed from the hatching baskets (using an egg separator), move over conveyer belts for selection, are vaccinated and manually sexed when necessary, counted and put in transport crates, after which they are moved to a holding room before transportation to the rearing farm. Chicken handling at the hatchery and transportation are both considered as potential stressors. Giersberg et al. (2020a) concluded that broiler chicken health, welfare and behaviour were only affected to a limited extent by hatchery procedures, as long as the drop height (<280 mm) and conveyer belt speed (<27 m/min) were not too high. Effects of transportation duration of chickens are ambiguous among studies (Valros et al., 2008; Bergoug et al., 2013; Jacobs et al., 2016; Hollemans et al., 2018).
- Removal of second-grade chickens. Second-grade chickens are normally removed in the hatchery during the selection process. Percentages of second-grade chickens vary among hatcheries and flocks. Van de Ven et al. (2012) found an average percentage of 1.15% second-grade chickens in both the conventional hatching and the Patio system. In on-farm hatching systems, second-grade chickens are not selected at the day of hatch, meaning that they will be present in the flock and selection should be done by the farmer in the first few days.
- Immediately access to feed and water immediately after hatching. In the last two decades, dozens of studies have been performed to study effects of early feeding. Recent reviews of specific aspects of early feeding highlight the main effects of early feeding of broiler chickens (De Jong et al., 2017; Taha-Abdelaziz et al., 2018; Jha et al., 2019).

Effects of alternative hatching systems on health, welfare and production

A number of studies have compared different alternative hatching systems with the conventional hatching system regarding effects on hatchability, chicken quality, mortality, performance, health and welfare (De Jong et al., 2019, 2020; Giersberg et al., 2020b; 2021; Jessen et al., 2021 a,b; Lingens et al., 2021; Souza da Silva et al., 2021). These studies showed ambiguous results, which can be largely related to differences in environmental and/or management factors, as described above. Because each study is performed at another place with different settings, it cannot be ruled out that findings are more related to the actual settings (e.g. temperature) of the different hatching systems than the hatching system itself.

Conclusion

Alternative hatching systems developed in the last 15 years differ in several aspects from the conventional hatching system. An important difference is the immediate access to feed and water after hatching, but other (climate) aspects, such as temperature play a role as well. Effects of different hatching systems on day-old chicken quality and health, welfare and performance are

ambiguous and appear to be related to (suboptimal) settings in both the conventional and alternative hatching systems. This suggests that circumstances in each hatching system are of more importance than the hatching system itself.

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OP09 The Effect of High Temperature of Incubation and Growing Period and Storage Conditions on Black Bone Syndrome in Broiler Legs

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Abstact

In recently, it has been reported that darkening of the leg bones of broilers was occurred and this color change also spread to the surrounding tissues after cooking process. Black Bone Syndrome (BBS) is defined as the presence of blood in the tissues around the tibia and femur bones, the redness of the meat color, and the darkening of the bone tissue in cooked legs. However, results in broiler processing plants and products on the market reveal that we are facing an important and widespread problem. This problem affects not only chicken meat product quality but also poultry welfare. Several studies have been carried out to determine the causes of the syndrome. In this study, it was aimed to research the effects of high temperatures applied during incubation and rearing periods of broiler chickens and keeping conditions of chicken thighs after the slaughtering process on the incidence of BBS. In research, cycling incubation temperature was applied during the incubation period, and chickens were exposed to high temperatures in the rearing period. On the 42 d old, without the gait-problem equal-sex broilers were slaughtered. BBS were assessed after the chicken thighs were kept for 3 days as fresh and 45 days as frozen. Heat treatment and sex were not found to be significant effects on BBS incidence. However, the storage conditions of the meat after slaughtering significantly affected the incidence of BBS. Freezing process increased the *incidence* of black bone syndrome (*BBS*) in drumsticks (tibia) and thighs (femur) of legs. It was determined that the *incidence* of BBS occurred at a higher level in the femur than in the tibia.

Keywords: Broiler, black bone syndrome, high temperature, storage conditions

OP10 Improving Bone Health of Broiler Chickens: Nutritional, Genotypic, Incubation and Activity Related Approaches

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Abstract

In the last decades, broiler chickens have undergone radical phenotypic and genotypic changes as a result of intense genetic selection for fast growth and high feed efficiency (Zuidhof et al., 2014; Tallentire et al., 2016). Accompanied by better management conditions and better feed quality, this selection has provided several advantages, such as high amount of meat production in a short time, less environmental pollution and considerable financial benefits for producers (Knowles et al., 2008; Zuidhof et al., 2014; Tallentire et al., 2016). However, it has also caused downsides for broiler chickens, such as poor leg health, impaired locomotion and lameness (Bradshaw et al., 2002; Knowles et al., 2008; Tallentire et al., 2016). One of the main reasons for these downsides is an imbalance between high growth rate and immature bones, because the speed of bone development is unable to keep up with fast growth. Impaired leg health causes serious welfare issues and considerable economic losses, since these chickens have difficulties accessing feed and water, suffer from pain, dehydration and starvation (Knowles et al., 2008; Sherlock et al., 2010; Shim et al., 2012; Pines and Reshef, 2015).

Based on these problems, the Healthy Bones project was established to investigate different approaches to improve leg bone health of fast- and slower-growing broiler chickens by (1) nutritional interventions in the broiler chickens or indirectly via the broiler breeders, (2) incubation conditions and (3) environmental enrichment. Within the project, five experiments were carried out, which will be explained in the following sections:

Results

Experiment 1 (Güz et al., 2019) investigated effects of a combination of organic macro and trace minerals, fish oil and hydrolyzed collagen in the diet of fast-growing male broiler chickens on tibia morphological, biophysical and mechanical characteristics (day 28, 35 and 42). Results of this study showed that organic minerals in the diet positively affected tibia morphological, biophysical and mechanical characteristics and also growth performance, while hydrolyzed collagen in the diet did not affect tibia characteristics and fish oil in the diet negatively affected these characteristics. It can be concluded that to improve bone development, more available minerals in the diet of current fast-growing broiler chickens might be needed.

Experiment 2 (Güz et al., 2021a) investigated effects of green LED light during incubation, and the separate effects of macro minerals source and trace minerals source during rearing on growth performance, tibia morphological, biophysical and mechanical characteristics (day 42) of fast-growing male broiler chickens. Results of this study showed that green LED light during incubation stimulated growth rate in later life, but did not influence any of the tibia characteristics. Organic macro minerals positively affected tibia characteristics compared to inorganic minerals and trace mineral source did not affect these characteristics. It can be concluded that green light during incubation stimulated growth performance in this study, but did not affect tibia characteristics. The source of macro minerals in the diet of broiler chickens seems to play a major role in bone development, rather than the source of trace minerals.

Experiment 3 (Güz et al., 2022) investigated effects of a combination of organic macro and trace minerals in the diet of fast and slower-growing broiler breeders on tibia morphological, biophysical and mechanical characteristics (at similar body weights) of their male offspring broilers. Results of this study showed that mineral concentration in eggs and hatchlings were hardly influenced by mineral source, but almost all tibia morphological, biophysical and mechanical characteristics of slower-growing offspring broilers were positively affected by organic minerals, whereas this effect was hardly seen in fast-growing chickens. It can be concluded that trans-generational mineral availability in offspring may play a role via other mechanisms than via absolute mineral concentrations in the egg. Moreover, the source of minerals in slower-growing

breeders' diet appears to be more effective on bone development of their offspring than in fast-growing chickens, which might be related to time available for bone development.

Experiment 4 (Güz et al., 2020) investigated effects of eggshell temperature pattern in week 2 (37.8°C or 38.9°C) and week 3 (36.7°C and 37.8°C) of incubation of fast-growing male broiler chickens on tibia morphological, biophysical and mechanical characteristics (day 41 or 42). Results of this study showed that a temperature of 38.9°C during the second week of incubation improved tibia characteristics of fast-growing broiler chickens. Incubation temperature in the third week appears to interact with the incubation temperature in the second week, resulting in the most advanced tibia development after incubation at 38.9°C in week 2, followed by 37.8°C in week 3 of incubation. It can be concluded that a 1.1°C higher EST than normal in week 2 of incubation seems to stimulate tibia morphological, biophysical and mechanical characteristics of broiler chickens. However, a 1.1°C lower EST in week 3 of incubation seem to have negative effects on tibia characteristics.

Experiment 5 (Güz et al., 2021b) investigated effects of pen enrichment consisting of ramps, platforms, perches, large distance between feed and water and provision of live Black Soldier fly larvae in the moss-peat dust bathing area on tibia morphological, biophysical and mechanical characteristics (at similar body weights) of fast and slower-growing male broiler chickens. Results of this study showed that pen enrichment positively affected tibia biophysical characteristics in both fast- and slower-growing chickens, while no effect was found on tibia morphological and mechanical characteristics. It can be concluded that pen enrichment can stimulate pathways involved in ossification and mineralization, rather than anatomical and physical bone properties.

Conclusion

Based on the results of this project, it can be concluded that (1) Replacement of a combination of inorganic macro and trace minerals by their organic varieties in the diet of fast-growing broiler chickens positively affected tibia characteristics. Same minerals broiler breeders resulted in better tibia characteristics in slower-growing offspring broilers, while no effect was observed in fast-growing offspring broilers. (2) Replacement of inorganic macro minerals in the diet of fast-growing broiler chickens by their organic varieties seems to be more effective than organic trace minerals on tibia characteristics. (3) Green LED light during incubation resulted in stimulated body weight gain, but no effect was found on bone development of broiler chickens. (6) An eggshell temperature of 38.9°C in second week, followed by 37.8°C in third week resulted in the most advanced tibia characteristics, while a 1.1°C lower EST in week 3 of incubation appears to have negative effects on tibia characteristics. (4) Pen enrichment positively affected tibia biophysical characteristics of both slower and fast-growing chickens, while no effect was found on tibia morphological and mechanical characteristics.

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OP11 Efffect of Egg Turning Duration and Frequency During Incubation on Hatchability

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Abstract

The field study was conducted that the effects of turning duration and the frequency during incubation on hatchability in a commercial hatchery. In this study, hatching eggs were collected from Ross 308 commercial broiler breeder flocks (37-55-wk-old). The eggs were either turned 24X/day during the first 14 d of incubation, and eggs were not turned during 15-18 d of incubation (Control) or were turned 96X/day for the first 11 day, and no turned during 12-18 d of incubation (Treatment). It was determined that increased turning frequency in the first 11 days caused a positive effect on the hatchability in 14 of 21 incubation batches ($P<0.05$). Consequently, these data demonstrated that hatchability was increased owing to 96X/day turning instead of 24X/day during the first 11 days of incubation.

Keyword: Turning frequency, turning duration, hatchability

IS[06] A Paradigm Change: Starch Digestibility in The Broiler Chicken

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Abstract

Starch digestibility in broiler chickens has been focused on young birds up to around 21 d of age. However, broiler chicken genetic lines have changed dramatically in the last decades and feed intake has increased a lot, especially in the grower and finisher diets. Svihus (2014) has shown a negative correlation between feed intake and starch digestibility. Based on these developments a number of studies have been conducted around the world showing the impact of age, resistant starch, and starch digestibility in different parts of the gastrointestinal tract and how amylase can impact animal performance and digestibility.

Keywords: poultry, performance, digestibility, starch, amylase

Introduction

Genetic development over the years : Historically, starch digestibility in broiler chickens has been focused on young birds up to around 21 d of age and some of these landmark publications are from several decades ago (Moran, 1982, Moran, 1985, Noy e Sklan, 1995; Noy & Sklan, 1997) when broiler chicken genetics were different from what we see today. In general, genetic lines select animals with high feed intake as this will impact body weight. Havenstein et al., (2003) demonstrated these differences by comparing a genetic line and nutrition approach from 1957 and 2001 (Table 1).

Table 1. Body weight (BW) at 56d and Feed Intake (FI) and Feed Conversion Ratio from 1 to 56d of age from two different genetic lines and nutritional approaches from 1957 and 2001 (adapted from Havenstein et al., 2003).

Genetic line and Nutritional Approach from:	BW at 56d (g)	FCR from 1 to 56d	FI from 1 to 56d (g)
2001	3946	1,96	7734
1957	809	2,54	2055

Patricio et al., (2012) found similar results in an overview of the performance of the Brazilian broilers chicken industry between 1990 and 2009 and found that in approximately 20 years, FCR was improved by 38 points which translates to approximately 2 points of FCR improvement per year.

Table 2. Overview of the broiler chicken performance in Brazil in 1990 and 2009 (adapted from Patricio et al., 2012)

Year	BW (g)	FCR	FI (g)	cFCR for 2.5 kg BW
1990	2061	2.058	4241	2.181
2009	2644	1.839	4862	1.797

It is important to also remember that body weight development and gut development are not linked, and this was proven by Zuidhof et al., (2014) where they compared different broiler chicken genetic lines and observed that gut weight at 56 d of age as a percentage of live body weight was 73% higher in the genetic line from 1957 (9.0%) than the 2005 broiler (5.2%). They also concluded that allometric analysis of gut yield data provided little evidence that commercial selection pressures have led to a change in gut yield. Jackson and Diamond, (1996), cited by Zuidhof et al., (2014), conclude that modern broilers have greater absolute gut mass than unselected birds. Selection for increased growth and efficiency does not increase digestive function or nutrient uptake per unit of gut mass; however, increased digestive and absorptive capacity in modern broilers is a function of increased gut size.

Gastro-Intestinal Tract Development during the life of broiler chickens : The digestive starch capacity in poultry increases as the intestinal tract matures and it is thought that this may be associated with an increase in pancreatic amylase production in older birds (Krogdahl & Sell, 1989). This has led to speculation that young animals may be particularly responsive to the augmentation of endogenous amylase systems with exogenous microbial amylases. However, when starch intake (especially

per unit intestinal tissue) is considered, it is actually older animals rather than younger who may benefit most from exogenous amylase supply. For example, Croom et al. (1999) noted that a 2-week old turkey chick had around four times more intestinal tissue per gram of body weight than a 16-week old turkey (Fig. 1).

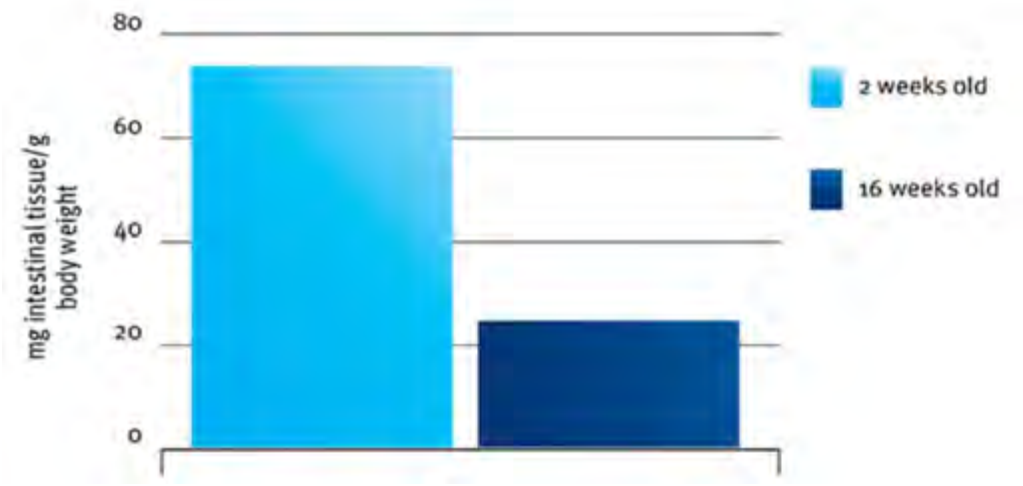

Figure 1. Effect of age on relative intestinal weight in turkeys (redrawn from Croom et al., 1999).

The paradigm changes

The relevance of these intestinal developmental changes relative to bodyweight gain for use of exogenous enzymes as augmentative agents may be counter-intuitive in that whilst the young chick has clearly a limited capacity to produce endogenous enzymes the intestinal tract accounts for a more substantial proportion of its body mass than is the case for a grower/finisher broiler. Thus, it may be more appropriate for supplementary enzymes such as amylase to be used in heavy broilers where the intestine and pancreatic tissue become an increasingly diminished proportion of the metabolic weight of the bird.

Aderibigbe et al., 2020a tested this hypothesis using broiler chickens fed a corn–SBM diet during 4 growth phases of day 0 to 11, 11 to 21, 21 to 42 or 42 to 56 after hatching in the presence or absence of exogenous alpha-amylase. The authors confirmed that amylase response for body weight gain and feed conversion ratio were higher after 21d of age (Figure 2) and this was linked to ileal digestible starch intake (Figure 3).

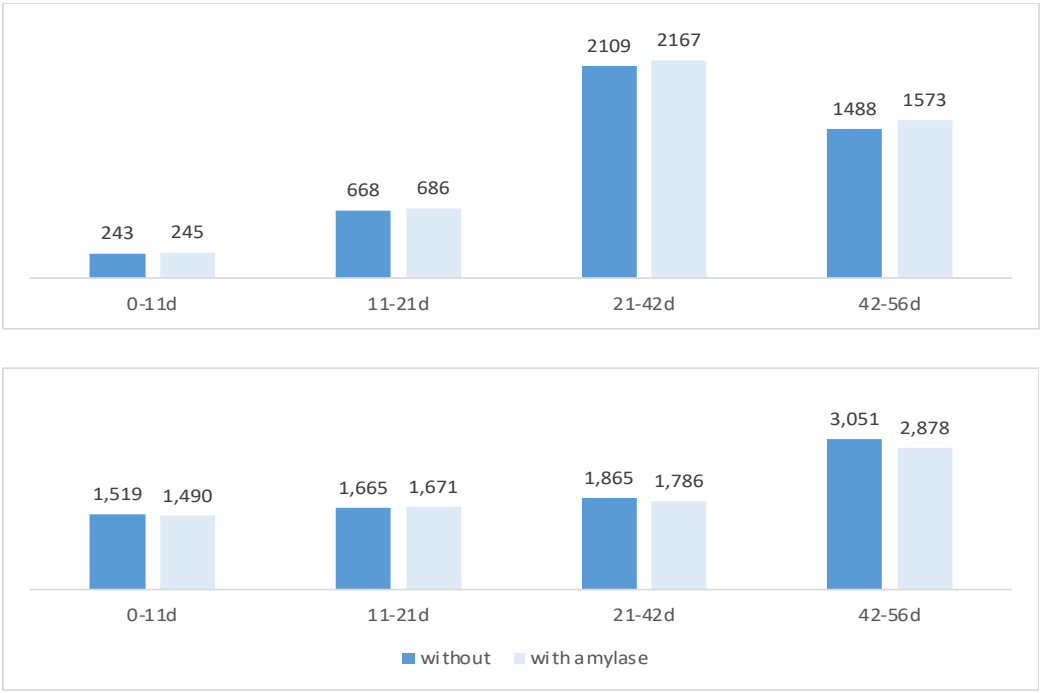

Figure 2. Effect of amylase supplementation (P<0.05) on body weight gain (in grams, top) and feed conversion ratio (bottom) of broiler chickens in different growth phases (adapted from Aderibigbe et al., 2020).

Supplementation of diets with exogenous amylase also shifts the site of starch and energy digestion to the posterior jejunum (Fig. 3; about 10% improvement from the anterior jejunum to posterior jejunum) suggesting a protein-sparing effect of the exogenous amylase by generating more sustained circulating levels of glucose to the lower small intestine, which would spare amino acids from catabolism and therefore increase feed efficiency and energy utilization (Aderibigbe et al., 2020).

In a dose-response trial with amylase in broiler chickens Schramm, et al., (2021) also observed a linear increase in resistant starch digestibility from ileal samples suggesting less starch in the hindgut and less substrate to be fermented by the microbiota (Weurding et al.,2003).

Gracia et al., (2003) and Ritz et al., (1995) may be the first to show the impact of a mono-component amylase in poultry, and publications with mono-component amylase are still scarce despite few new publications in recent years (Stefanello et al. 2015, 2017, 2019; De Faria Castro et al. 2020; Yin et al. 2018; Vieira et al. 2015; Jiang et al. 2008; Onderci et al., 2006; Cordova et al., 2020, 2021, Kaczmarek et al., 2014, 2022, Perz et al., 2022, Gernaey et al., 2018, Liu et al., 2020, Yuan et al., 2017, Zhou et al., 2021, Schramm et al., 2021, Aderibigbe et al., 2020a,b).

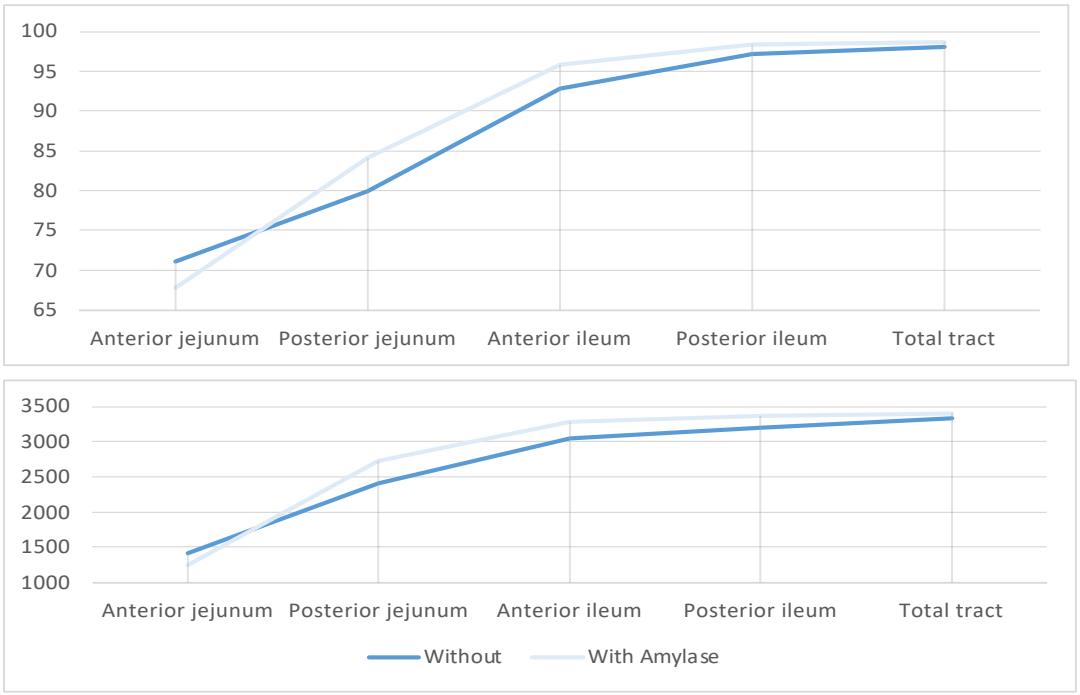

Figure 3. Effect of amylase supplementation on starch digestibility (%, top) and digestible energy or metabolizable energy (kcal/g, bottom) in different intestinal sites of the broiler chicken (adapted from Aderibigbe et al., 2020).

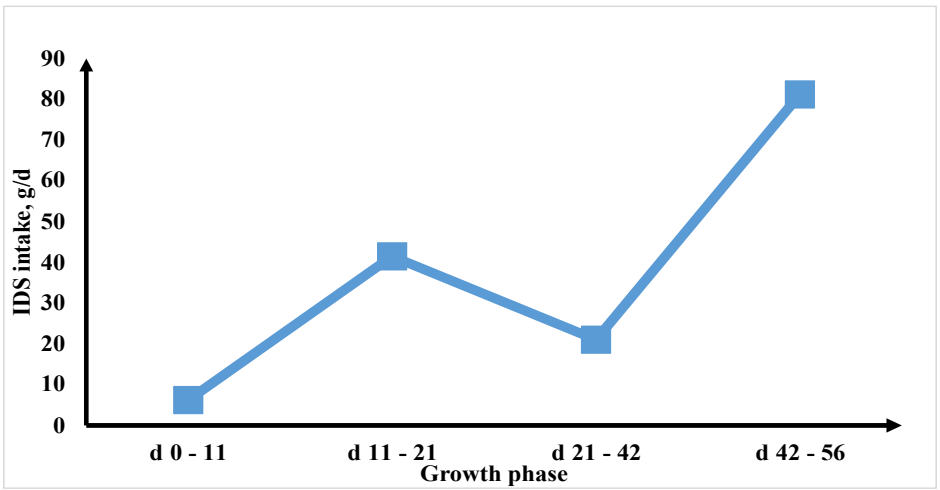

Figure 3. Changes in ileal digestible starch (IDS) intake of broiler chickens in the four growth phases as a result of α-amylase supplementation. Square data points represent the mean α-amylase effect on IDS intake, relative to the control diet, in the four growth phases (Aderibigbe et al., 2020).

Conclusion

Even though starch digestibility in poultry is high, it is possible to conclude that alpha-amylase has a very good potential to improve performance or reduce diet cost, especially after 21 d of age. Furthermore, exogenous amylase may have a direct impact on energy release from starch with a very different mode of action from other NSP enzymes where the main mode of action is linked to nutrient encapsulation, viscosity, and an in situ prebiotic effect. Further work is required to optimize amylase dosing and to more fully understand heterogeneity of starch digestion across individual birds, especially for diets with non-conventional starch sources. Despite some persistent knowledge gaps, augmentation of endogenous starch-digesting infrastructure with an exogenous amylase is an effective strategy to reduce variance in energy supply to broilers and to enhance live production outcomes.

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OP12 The Effect of Guanidinoacetic Acid Supplementation Applying Reduced Energy Provision Under Commercial-Scale Research Conditions

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Abstract

Creatine (Cr), together with its direct precursor guanidinoacetic acid (GAA), improves cellular energy and is consequently a feed additive worth considering counteracting high energy donor prices. This study aimed to determine the effects of GAA provision on growth performance given reduced energy levels in a commercial broiler diet. A total of 1088 Ross 308 AP day-old male chickens were allotted to four treatments, with eight repetitions of 34 chickens per treatment. The experimental design was completely randomized and the treatments were as follows: T1: Control; T2: Negative control (-80 kcal for starter and grower/finisher); T3: T1 + 600g GAA on top; T4: T2 + 600g GAA. Overall T4 not only compensated for the reduced energy provision, but provided additional weight gain and feed conversion ratio (+ 21g and -2 points, respectively), while T3 provided 59 grams additional weight gain and 4 points less in feed conversion ratio. Overall, GAA is an effective nutritional additive to spare dietary energy and it can be used with full flexibility according to various regional production goals.

Introduction

Nutritional additives are widely used to improve economic efficiency in modern poultry production. While the prices of energy donors continuously rise, nutritionists pay special attention to strategies, which alter the provision of dietary energy in order to improve overall energy efficiency. Guanidinoacetic acid (GAA) as the single precursor of creatine (Cr) is present in the body of all vertebrates. Unlike other nutrients, together with phosphocreatine, it directly recharges adenosine-triphosphate for immediate energy supply. Therefore, Cr plays a vital role in energy metabolism and in the formation of muscle cells as well as other body tissues (Brosnan *et al*. [1]). As the energy contribution of GAA provided as a nutritional additive takes place on a cellular level it is also independent from other feed supplements enhancing the digestibility of raw materials such as enzymes or emulsifiers. Scientific trials established an energy equivalence of 0.06% GAA to 50-100 kcal (0.21-0.42 MJ) AME_N per kg final broiler feed, depending on production goals. Consequently, to be in line with local production settings, the present study was planned to evaluate the effects of GAA provision on growth performance given specifically reduced energy levels of the present broiler diets in a commercial-scale research house.

Materials and Methods

The study was carried out at the experimental farm of Italcol, located in the department of Santander-Colombia at an altitude of 1,005 meters above sea level and lasted 35 days. A total of 1088 Ross 308 AP day-old male chickens with an initial body weight of 42.5 ± 1.05g, were allotted to four treatments, with eight repetitions of 34 chickens per treatment. The experimental design was completely randomized. The treatments were: T1: Control; T2: Negative control (-80 kcal for starter and grower/finisher); T3: T1 + 600g GAA on top; T4: T2 + 600g GAA. The trial comprised of three phases: The pre-starter phase was defined up to a consumption of 250 g/bird, the starter phase up to a consumption of 850 g/bird and the grower/finisher phase up to day 35 of age. The diets were offered in broken form for both pre-starter and starter phase and in pelleted form during the grower/finisher phase. The following production parameters were determined/calculated: Feed intake, body weight, feed conversion ratio, weekly survival and European Efficiency Index (EEI). After verification of the assumptions of homogeneity of the variances and normality of error, the data were subjected to ANOVA analysis of variance, with a significance level of 5%. When significant differences were found ($P < 0.05$), the means were compared using the Tukey test. For data analysis, the statistical program JMP version 15.2 was used.

Results and Discussion

On day 35, when we compared the group supplemented with 600g/ton of GAA considering an energy matrix of 80 kcal, with the control group, we found that the GAA group obtained 21 grams more per chicken and 2 points less in feed conversion ratio ($p < 0.05$). When we compared the group supplemented with 600g/ton of GAA on top with the control group, we found

that the GAA group obtained 59 grams more per chicken and 4 points less in feed conversion ratio ($p < 0.05$).

Conclusion

To sum up, GAA is an effective nutritional additive to be applied in reduced energy diets in order to meet or exceed (in terms of other productive parameters) the given settings. In this study the use of GAA considered with a matrix value of 80 kcal at 0.06% supplementation level improved productive performance in addition, while the on top application reached its full potential.

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OP13 Can Betaine Alleviate the Effects of Heat Stress in Broiler Chickens?

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Abstract

This study was planned to investigate the effects of adding betaine and/or choline to broiler diets subjected to heat stress after 25 days of age. A total of 765 day-old male Ross 308 chicks were randomly distributed into six dietary treatments each has eight replicates with seventeen chicks in floor pens until 42 days of age. The basal diet (TR1) with no added betaine or choline (control); TR2, added betaine 700, 600, 500 mg/kg and TR3, added choline 700, 600, 500 mg/kg at starter, grower and finisher feeds respectively; TR4, added betaine 1000 mg/kg feed and TR5, added choline 1000 mg/kg feed at all periods; TR6, added betaine and choline 500 mg/kg feed at all periods. Betaine supplementation has significantly improved the growth performance of broilers especially under heat stress compared control ($P<0.05$). However, there was no significant difference between the doses of betaine and choline ($P>0.05$). Carcass and digestive organ parameters was not significantly influenced by either betaine of choline addition ($P>0.05$). Oxidative status of liver and breast meat were significantly improved by the addition of betaine, but with higher doses of choline ($P<0.05$). The best jejenum growth was obtained with lower dose of betaine compared to control and other treatments ($P<0.05$). The heat shock protein expression (HSP70) in liver increased with the both level of betaine, while only higher dose of choline was effective ($P<0.05$).

Introduction

In the current century, combating heat stress remains a challenge for the broiler industry in the tropics, and efforts are under way to develop dietary measures that can ameliorate heat stress to achieve optimum performance. Exposure to high ambient temperature causes serious physiological dysfunctions and triggers secretion of corticosteroids (1), which being catabolic in nature severely depress animal performance. By elevating circulatory corticosteroids and decreasing thyroid activity, heat stress impairs broiler performance. Drastic decline in feed intake occurs in heat-stressed birds as a physiological response to minimize intrinsic heat production and to maintain the thermal homeostasis, thus bringing down feed efficiency, live weight gain, and survival rates. High ambient temperature causes a decrease in protein and amino acid digestibility (2, 3). Betaine, the trimethyl derivative of the amino acid glycine, is a naturally occurring compound distributed widely in many plants and animal tissues. The chief physiological role of betaine is to function as a methyl donor and an osmolyte. Betaine also acts as an osmolyte, to maintain the avian's cellular water and ion balance to improve the avian's capacity against heat stress via preventing dehydration and osmotic inactivation. Dietary supplementation of betaine presumably reduces the requirement for other methyl-group donors, such as methionine and choline. Among the potential benefits of its inclusion in poultry feeds are sparing choline, carcass fat reduction and aiding cell osmoregulation (4).

Materials and Methods

Birds and housing: The research was carried out in the Poultry House of Animal Science Department, Ankara University. 765 day-old male Ross 308 chicks were used as animal material. At the beginning of study (day 1), chicks were weighed and randomly allocated to 45 floor pens (1.20*0.95 m =1.14 m²), littered with wood shavings and equipped with nipple drinkers and plastic hanging poultry feeders manually filled. The light program and heating via automatic heating, cooling and ventilation system was consistent with the Ross Broiler Management Manual (5). Cyclic heat stress will be started when birds are 25 days old, 6 hours at 32.5±1 °C during day time, approximately between 12.00 am and 6.00 pm by an automated heater, and 3 hours of decreasing temperature down to 24 °C (6).

Experimental design and treatments: The research was conducted according to completely randomised block design. Day old chicks were randomly distributed into 6 dietary treatments each has 8 replicates with 17 chicks in floor pens. The basal diet (TR1) with no added betaine or choline (control); TR2, added betaine 700, 600, 500 mg/kg at starter, grower and finisher feeds respectively; TR3, added choline 700, 600, 500 mg/kg at starter, grower and finisher feeds respectively; TR4, added betaine 1000 mg/kg feed at all periods; TR5, added choline 1000 mg/kg feed at all periods; TR6, added betaine and choline

500 mg/kg feed at all periods. The basal diet was formulated to contain 2990, 3095, 3190 AMEn kcal/kg; 23.22, 21.23, 19.4% crude protein, 1.28, 1.15, 1.03 digestible lysine, 0.95, 0.87, 0.80% digestible methionine+cysteine and 1421.1, 1322.9, 1232 mg/kg choline at starter, grower and finisher feeds respectively. Chicks were allowed ad libitum access to water and mash feeds. Betain used in the experiment (ActiBeet® 96) was supplied by Agrana Company (Vienna, Austria). ActiBeet® 96 contains 96% betaine as betaine anhydrate plus anticaking agent. Choline chloride (75 %) was supplied by a local broiler entegration from a fresh batch of tank and contains 560.000 mg choline/kg.

Feeds and diets and nutrient analysis: Experimental diets based on corn and soybean meal was introduced during starter (0-10 days), grower (11-24 days), and finisher (25-41 days) periods. The diets were formulated according to nutrient recommendations of Ross 308 (7). The crude nutrients in feedstuffs and mixed feeds were analysed for proximate (8), amino acid and AMEn content by near infrared reflectance spectroscopy (NIRS; Evonik Nutrition & Care GmbH, Hanau, Germany).

Measurements of growth performance: Live body weight (BW) and feed intake (FI) were measured on days of initial (0), 11th, 25th 35th, and 42nd days of experiment. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for 0-10d, 11-24d, 25-35d 35-42d and 0-42d. All measurements were made on for each replication on a pen-base. Mortality was recorded daily.

Carcass and organ measurements: At the end of experiment, sixteen birds per treatment were randomly picked out (2 per pen) after an overnight (8 h) fast, weighed. Each bird was exsanguinated by cutting the jugular vein, allowed to bleed for approximately 2 min, scalded for 30 s, and defeathered in a rotary picker. Viscera and abdominal fat was removed. Carcass yield, abdominal fat, some digestive organs, thighs, drumsticks and breast meat were weighed and calculated as a fraction of individual live body weight.

Determinations of malondialdehyde (MDA) levels in tissues: Liver and breast muscle samples were homogenised in 10% (w/v) physiological saline on ice for 60 s and then sonicated with an ultrasonic wave cell grinder (NingBo Scientz Biotech. Comp.) for 1 min (on 1s, interval 2s). The homogenates were centrifuged at 1000 g for 15 min at 4°C and supernatants were collected to determine MDA levels. The levels of MDA in the supernatant was determined using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute) and expressed as MDA content per mg protein.

RNA extractions (Heat Shock Protein 70): Total RNA was isolated from liver samples using Trizol reagent (15596018; Life Tech.) according to the manufacturer's instructions. The concentration of each isolated RNA sample was determined using a NanoDrop Spectrophotomter (ND-2000; Gene Company Ltd) and the integrity of the RNA was checked using denatured RNA electrophoresis.

Histological analysis of jejenum: For the histological examination, segments of 5 cm long were taken from the midpoint of the jejunum and flushed with a saline solution. These segments were fixed in 10% neutral buffered formalin at room temperature for 72 hours. After the routine histological procedures, samples were embedded in parafin wax. Sections having a thickness of 4 μm were taken on the glass slide using a microtome. The variables measured were villus height and width, villus height to crypt depth ratio and villus surface area (9).

Statistical analysis: The data for all response variables were analysed as a completely randomized block design by using General ANOVA/MANOVA procedure of the Statistica (1984). When significant differences ($P < 0.05$) among groups are found, means were separated using the Tukey HSD test. The data of mortality were subjected to quare root transformation and analysed by one-way ANOVA.

Results

Overall, the results of the present study showed that efficacy of betaine supplementation in broiler diets has significantly improved the growth performance of broilers especially after starter periods compared to the unsupplemented control birds ($P<0.05$). The effect of betaine was more clear after grower period during heat stress ($P<0.05$). However there was no significant difference between the doses of betaine supplementation ($P>0.05$). Besides, there was also no significant differences between betaine and choline supplementation. Carcass and digestive organ parameters as percentage of live body weight and mortality was not significantly influenced by either betaine of choline addition ($P>0.05$). Oxidative status of liver and breast meat were significantly improved by the addition of both doses of betaine, while only higher doses of choline (1000 mg in all periods) and betain+choline (500+500 mg) had an improvement in liver MDA level compared to control ($P<0.05$). This situation was also supported with the results of jejenum morphology. The best jejenum growth was obtained with lower dose of betaine (700 600, 500 mg at starter, grower and finisher respectively) when villus height (VH), crypt depth

(CD), VH/CD and goblet cell numbers of jejenum were considered compared to control and other treatments (P<0.05). Lower inclusion of choline was not as good as betaine to support the jejenum epithelial development. Increased dose of betaine had no additional benefit compared to the lower inclusion of betaine, while had better results compared to the control. The heat shock protein expression (HSP70) in liver increased in the treatment groups except lower choline supplementation (P<0.05).

Conclusion

The supplementation of diets with betaine improved broiler performance, jejenum growth and oxidative status of broilers under heat stress. As seen in liver MDA level and jejenum development, lower inclusion of choline was not also good enough to alleviate the stress of heat. However, it was interesting to see the synergetic effect of 500 mg betaine and 500 mg choline by doubling the expression of HSP70 gene. So it can be concluded that betaine and choline has different mode of action and even lower dose of betaine had positive effect of growth performance of broilers with the absence of supplemental choline chloride, and very helpful to alleviate the negative effects of heat stress.

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OP14 The Use of Different Selenium Sources and Vitamin E Dosages As a Strategy to Enhance The Performance of Broilers Raised in a Tropical Zone

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Abstract

Broilers raised in a tropical climate show a higher propensity to suffer challenges to their antioxidant systems due to heat stress, thus hampering their performance. Vitamin E and selenium (Se) are essential nutrients playing a key role on the antioxidant system and they are often supplemented in animal feed. Dietary Se supplementation is mainly using either inorganic form such as sodium selenite (SS) or organic forms such as pure forms of selenomethionine (SeMet) products. Hydroxy-selenomethionine (OH-SeMet) is a chemically synthesized organic Se form and has been proven to be highly efficient in transferring Se from diet to muscle compared to SS. The transfer of Se in tissues, in fact, leads to the buildup of a Se reserve as SeMet in the body that would participate to maintain an effective antioxidant defenses and enhance poultry performances, particularly during critical periods. The objective of this study was to evaluate the use of different selenium sources and vitamin E dosages as a strategy to enhance the performance of male broilers raised in a tropical zone. The treatments were distributed in a 2 x 2 factorial design: two selenium sources (OH-SeMet or SS) and two dosages of vitamin E (30 ppm or 60 ppm) that provided 4 treatments with 10 replicates of 20 birds each, for a total of 800 broiler chickens (Cobb 500) having an average day-old body weight of 42 g. They were housed on litter in 1.5m x 1.5m boxes, with tubular feeders and pendulum dirking and fed *ad libitum*. In this trial, corn and soybean meal-based diets were distributed in 3 phases: 1 to 21, 22 to 34, and 35 to 44 days. One–to-44-day performance was evaluated by analysis of variance (ANOVA) ($P<0.05$). There was significant interaction between selenium sources and vitamin E doses for feed intake ($P<0.001$) and feed conversion ratio ($P=0.001$). No interaction was observed between these factors for body weight gain ($P=0.254$). For feed conversion ratio, when 30 ppm of vitamin E was added to diets, no difference between selenium sources was observed (1.626 OH-SeMet vs 1.644 SS; $P=0.189$). The addition of 60 ppm of vitamin E showed the best feed conversion ratio at 44 days of age in the OH-SeMet treatment (1.588 OH-SeMet vs 1.682 SS; $P<0.001$). Selenium sources affected body weight gain. The highest body weight gain was observed in OH-SeMet-fed birds, regardless of vitamin E dosage (3.307 kg OH-SeMet vs 3.173 kg SS; $P<0.001$). On the other hand, vitamin E levels did not optimize this production parameter since the increase from 30 to 60 ppm did not affect body weight gain (3.244 30 ppm vs 3.235 60 ppm; $P=0.531$). These results show that OH-SeMet provides greater balance to the birds' redox system and generates benefits for the performance of male broilers raised in environments predisposed to heat stress. In summary, thanks to its higher bio-efficacy, the use of OH-SeMet combined with a higher vitamin E dosage in broilers raised in a tropical zone is the best strategy to mitigate the effects of heat stress.

IS07 Adenovirus Infections In Poultry - Control and Diagnosis

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Adenovirus, Related Problems and Control

Adenoviridae family is widespread in humans and animals. The first isolations had been made in the early 1950s. from surgically removed tonsils in children, and then from the word adenoids (glandular lymphatic tissue), they were named Adenovirus. In the following years a several adenovirus strains were isolated from mammals, birds, fish, amphibians and reptiles. The International Committee on Taxonomy has grouped Adenoviridae into two types: Mastadenovirus- viruses of mammals and Aviadenovirus - responsible for infections occurring in birds. Avian viruses are different in antigenic and gene structure from mammalian viruses; however, their physicochemical properties are the same.

Adenoviruses isolated from birds are classified into the following genera: Atadenovirus, Aviadenovirus and Siadenovirus. Adenoviruses are non-enveloped viruses with a capsid size of 70–90 nm. The unsegmented genome of these viruses constitutes linear dsDNA containing genes encoding structural and non-structural viral proteins. The DNA size of adenoviruses is 25–43.8 kbp. and constitutes 11% to 17% of the total virion. The main capsid protein of adenoviruses is hexon. The molecular weight of this protein is 103 kDa. This protein is highly variable and contains various antigenic determinants responsible for serological differentiation of all adenoviruses. The structure of the hexon contains conservative domains, that form the basis of the protein, as well as highly variable domains that are arranged outside the virion and are responsible for antigenic variability.

The tropism of adenoviruses for bird tissues depends on the virus strain and its pathogenicity. The question is whether these viruses are commensal or pathogenic. These viruses are common in poultry flocks, they can be isolated from fully healthy birds, but their role in causing several dangerous disease syndromes in broiler chickens is also well documented. Both vertical and horizontal transmission play a role in the spread of adenoviruses. Horizontal transmission occurs through contact with the mucous membrane of the beak and nasal cavities with contaminated litter, feed or water, as well as direct contact with infected birds.

Replication of adenoviruses takes place in the nucleus of infected cells. It begins when the virus enters the host cell, then the viral DNA is transferred to the cell nucleus, where the processes of transcription and translation take place. The process of accumulation of viral proteins, and then their formation into complete virions, is completed in the cell nucleus, which are destroyed with the release of virus's particles.

The mechanisms of differential pathogenicity of adenoviruses are not fully understood. Strains belonging to the same genotype often show different pathogenic properties. Domestic fowl species are susceptible to infection at any age.

Avian adenoviruses have been divided into three groups. Group I includes adenoviruses found in chickens, turkeys, ducks and geese. Based on molecular analysis with restriction enzymes, group I adenoviruses were differentiated into 12 serotypes (FAdV1 - FAdV12) occurring in chickens, ducks, pigeons and turkeys and 5 genotypes A-E.

Adenovirus are the etiological factor of many diseases in chicken broilers, such as:

- Inclusion Body Hepatitis–IBH;
- Gizzard Erosion and Ulceration–GEU;
- Hydropericardium Hepatitis Syndrome–HHS (Angar's disease).

Currently, it is assumed that some types of FAdV (2, 11/D, 8a and 8b/E) may be responsible for the clinical symptoms of IBH, while strains belonging to the type/species of FAdV-1/A are mainly responsible for causing Gizzard Erosion, and strains 4/C are responsible for Angar's disease. What is very important in the diagnosis of adenovirus infections, should be considered the possibility of infection of one bird with different species/types of the virus.

A special feature of adenoviruses is their high capacity for extensive and frequent vertical transmission.

Layers infected during production shed adenoviruses to eggs for 5-9 weeks. If immunity is developed shedding with this serotype stops, but the virus does not disappear from the birds. Adenoviral infections can have a latent course and stress, hormonal disorders - can lead to reactivation and the appearance of clinical symptoms of re-shedding. Characteristic is the lack of cross-protection after infection/vaccination, antibodies after contact with one type of virus do not protect against infection with another type.

In the diagnosis of adenovirus infections in broiler chickens, it is essential to answer the question whether the infection occurred by horizontal or vertical infection? Due to the responsibility of the hatchery, which, according to the contract, should deliver chicks free of vertically transmitted diseases, it seems that the presence of FAdV infection causing losses in vertically infected broilers should be compensated by the supplier of chicks. After infection in natural conditions, the incubation period of adenovirus infections is very short (24-48 hours), that leads to the rapid spread of infection in the infected herd. In broiler chicken flocks, symptoms of adenovirus infections appear most often in the 2-3 week of life. In field conditions, the course of infection is influenced by many factors, including co-infections, especially with immunosuppressive viruses (Marek's disease, infectious chicken anemia, Gumboro disease or reoviruses). In the case of adenovirus infections of broiler flocks, diagnostics is very important and at the same time complicated.

In the *Anamnsesis* of the disease should be include questions regarding the age of symptoms, the number of affected birds, the dynamics of the spread of the disease in the flock, mortality, suppliers of chicks, etc. Because the shedding of adenoviruses by the parent herd lasts even several weeks; therefore, during the *Anamnesis*, we must try to determine whether the offspring are from the same parent herd that had similar symptoms. In routine laboratory diagnostics of adenovirus infections, the principle of the golden diagnostic triangle is used, i.e. molecular, serological and histopathological tests. In the epidemiological investigation, the cultivation of the virus in chicken embryos along with the typing of the virus is very important. The PCR technique is used in molecular diagnostics. In the conventional method, a product with a size of 830 bp is searched for in the material taken from diseased sections. However, in the quantitative polymerase chain reaction qPCR (Real time PCR) we have additional information in the form of the ct value, that informs us about the potential number of virus particles in the tested samples. For a proper assessment of the pathogenic role of adenoviruses, it is necessary to perform a histopathological examination. The results of these tests are the only reliable evidence confirming the severity and nature of pathological changes caused by viruses in internal organs. In most diseases caused by avian adenoviruses, intranuclear inclusion bodies are formed in parenchymal organs. Histopathological reveals characteristic Cowdry type-A inclusion bodies. It should be noted that inclusion bodies appear 5-7 days after infection and may be difficult to detect after several days. Serological tests are also a great diagnostic tool. Commercial ELISAs are routinely used, but SN and agarose gel precipitation are also of great importance. In ELISA, very high levels of antibodies are induced after adenovirus infection. However, it is not possible to say with certainty whether they came from contact with a virulent or commensal FAdV strain. Virus isolation of group I adenoviruses is carried out in chicken embryos or in cell cultures. Appropriately selected cell cultures are used depending on the clinical symptoms and autopsy changes. The most useful and susceptible to infection of this group adenoviruses are cultures of chick embryo liver (CEL) cells and chick embryo kidney cells (CEK).

Historically, the first published cases of IBH in Poland were recorded in the 1980 (Karpinska et al.). The description of a case of gizzard erosion was recorded in 1993 (Borzemska et al.). The real eruption of IBH and GE occurred in 2007. At that time, there were visible problems with routine diagnostics and, most importantly, the lack of information flow from poultry hatcheries. At that time, lesions appeared after the 7th day of life of chicks and occurred mainly in the liver and kidneys. The diagnostic procedure carried out at that time suggested that the reason was "poisoned feed". The treatment at that time was feed replacement, which was associated with a decrease in mortality related to the course of the disease - that was the key to diagnosis. In addition, the lack of symptoms in the parent flocks confirmed the story of "poisonous feed" and the symptoms occurred after the first week of life, so there was no link to the parent flock.

The first signals that it is not feed poisoning came from the results of histopathological tests. The presence of characteristic inclusion bodies caused that culture examinations were performed in various centers in Europe (Vienna, Deventer, Cuxhaven, Budapest). The result came after a few weeks and was devastating. Adenoviruses were found in most of the tested samples. Additional information was the analysis of the correlation of cases in broilers with parent flocks.

The first cases of GE came a few months later. In mid-2008, cases were reported with the characteristic "black ingesta" in the crop and stomach. Changes appeared around 8 days of life. At the time the diagnostics revealed 'poisoned feed' pointing out to high levels of histidine However, the help of experienced colleagues from other countries resulted in a series of tests that confirmed the presence of adenoviruses. In the period from September 2007 until December 2008 a total of 278 cases were

analysed. All the cases were confirmed by PCR as positive. Of these cases, 169 were decided to undergo viral culture testing. Of the 169 viral culture tested, only 75 were confirmed as positive. From the obtained positive cultures, the typing process was carried out, in which the presence of species A, B, D, E was confirmed. The most frequently isolated serotype from GE cases was serotype 1, and from IBH cases, 1, 5, 8, 11. Detailed data on the identified variants are presented in the chart below.

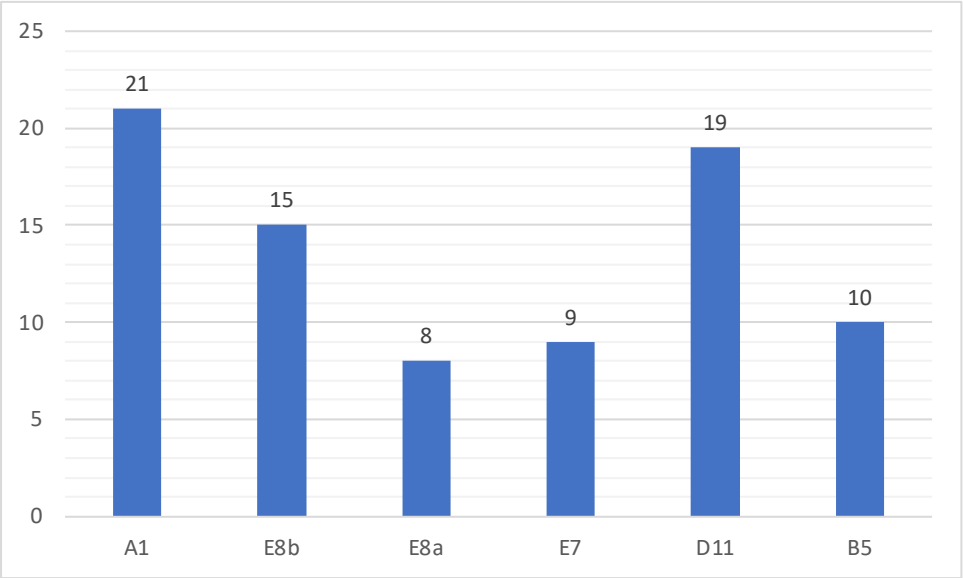


Figure 1. Species/serotypes of isolates found in 2007-2008 from IBH and GE. A total of 82 species/serotypes were identified as two different species were isolated from one case in several samples.

In our laboratory, over the course of 2020, the number of reported needs for molecular diagnostics of FADV infections accounts for about 14% of all PCR tests performed. In 2020, a total of 279 samples were tested by PCR for the presence of FADV DNA. Of the 279 tested samples, 108 samples were positive, which is 39% of all samples tested in this direction. Detailed data on PCR diagnostics of adenovirus infections are presented in the chart below.

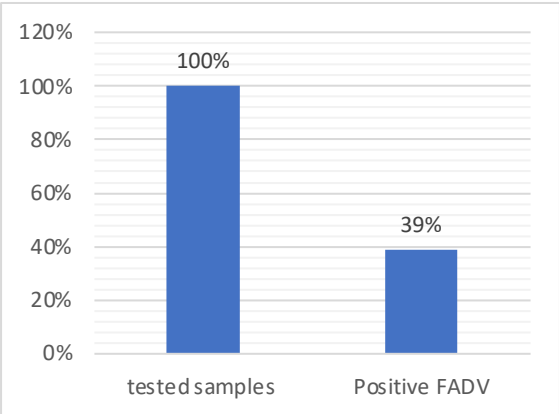


Figure 2. Percentage of positive samples for FADV in 2020.

When analyzing the results obtained from different types of poultry production in 2020, the most positive samples were found from broiler chickens, i.e. 67% all positive samples, whereas from the rest type of poultry 34% (broiler parents and chicken comercial layers). Most of the cases were associated with the occurrence of IBH (46%), the rest were associated with GE (30%) and the presence of the virus itself without any obvious disease symptoms (24%).

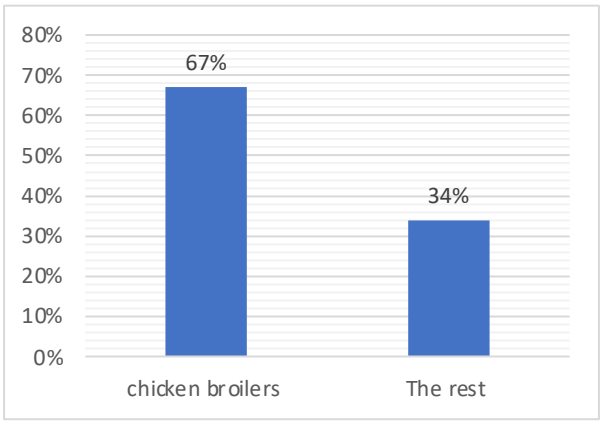


Figure 3. Percentage of positive FADV samples from broilers and other poultry species in 2020.

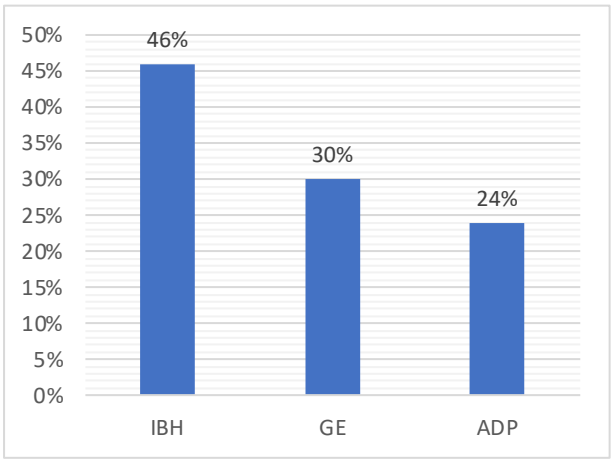


Figure 4. Percentage of clinical cases of adenovirus in broiler chickens in 2020.

When we analyze the occurrence of positive results isolated from broiler chickens in different ages, we find that the most positive results were obtained from birds at the age of 4 weeks of life (18). The smallest numbers of positive samples are from the first week of life birds (2).

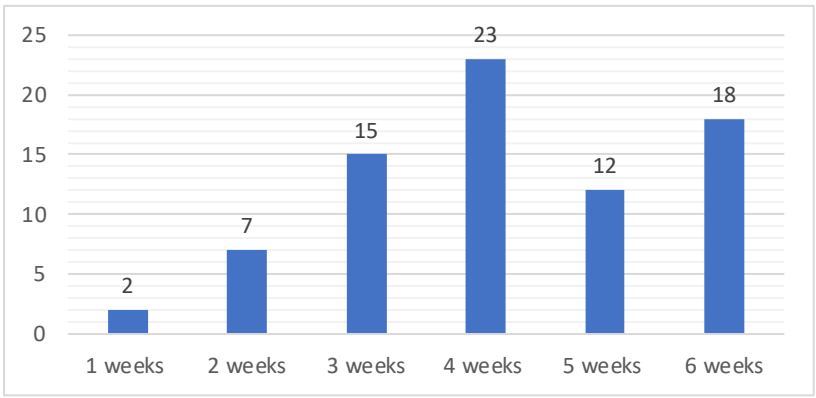


Figure 5. Numbers of positive FADV samples isolated from broiler chickens in different ages in 2020

Of the broiler chicken samples tested in 2020, 49 have been typed. The most frequently reported species/serotypes were B5 and D11. Detailed data on species and types found in 2020 are presented in the chart below.

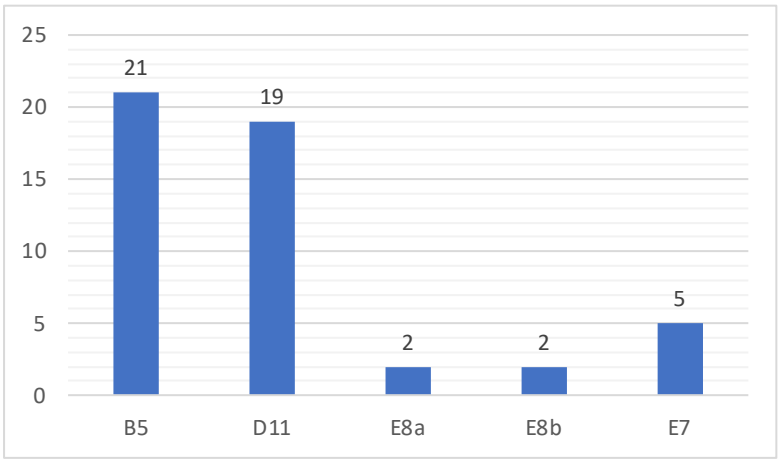


Figure 6. Species/serotypes of isolates found in 2020.

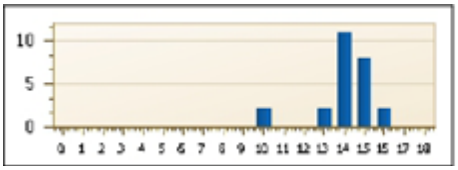
However, it seems that the most important and difficult task for a veterinarian is not the diagnosis of FAdV infection, but the determination of the economic losses caused by the infection. It is very important to correctly diagnose co-infections that can increase losses. In a large integration case study, it was found that FADV infections significantly worsened production rates; Two large production companies located far from each other, with separate staff and equipment; The chicks supplier was the same as the feed producer; The biggest problems noticed on one of the farms were high feed consumption and relatively poor growth.

Detailed production data is presented in the table below (Table 1).

A good tool to control FADV infections in broiler flocks is the use of autogenous vaccines in parent flocks. In this case, material from clinical cases should be taken from broiler chickens and subjected to a multiplication process. Then, the material should be typed in order to determine the species and serotype. It should be remembered that there is no cross-protection, so proper diagnosis is very important to determine all possible species/serotypes. A mistake made at this stage will make the situation worse. The autovaccine is an inactivated product used in injection, usually intramuscularly. Serological methods (ELISA) are used in the control of autovaccines. They are used both to control the quality of the autovaccine itself and the injection process. Below are the sample histograms from ELISA tests (Biochek) of serum samples taken from broiler parents.

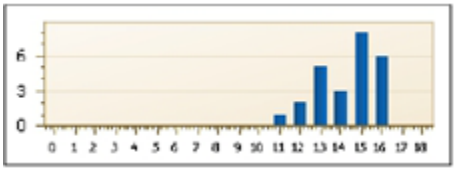
Table 1. Selected production parameters in a broiler flock with Adenovirus B5 isolated from internal organs compared to a healthy flock.

Production factor	Farm with problems	Farm without problems
Number of birds	46 00	44 000
Mortality and selection	6,7	3,8
Average body weight (kg)	2,67	3,11
Production days	46	42
FCR	1,89	1,49
Additionally identified problems	Coccidiosis positive vvIBD in PCR at 38 days of age	



	Assay:	FAV1		Lot number:	FS 5532
	Sampling date:	2013-06-04		Test date:	2013-06-06
				No. samples:	25
Mean	Titer:	24478	%CV: 19	VI:	1288
Target	Titer:		%CV:	VI:	
Interpret	Titer:		CV:	VI:	

Figure 7. ELISA serum samples results from broiler parents 24 weeks of life, vaccination at 18 weeks.



	Assay:	FAV1		Lot number:	FS 5532
	Sampling date:	2013-05-27		Test date:	2013-05-29
				No. samples:	25
Mean	Titer:	25313	%CV: 20	VI:	1265
Target	Titer:		%CV:	VI:	
Interpret	Titer:		CV:	VI:	

Figure 8. ELISA serum samples results from broiler parents 26 weeks of life, vaccination at 18 weeks.

In practice, the diagnosis of the of adenovirus infections is extremely complicated and the criteria used as a diagnostic tool are fluid and often subjective. A very important issue determining the diagnostic success is the cooperation between the vet taking care of the broiler flock and the poultry hatchery. This affects not only the diagnostic process itself, but also the prophylactic procedures related to the production of autovaccines for broiler parents. However, the most difficult challenges that the future poses for us are: determining the key to assessing the virulence factors of adenovirus strains and establishing the impact of co-factors, that are essential for the occurrence and the course of the disease in the herd.

OP15 Surveillance of Avian Coronavirus Infectious Bronchitis Virus, Infectious Laryngotracheitis Virus, Avian Metapneumovirus and Avian Reovirus in Poultry Flocks with Respiratory Tracts Symptoms in Türkiye

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Disclaimer: (This research paper is already published in the "Turkish Journal of Veterinary & Animal Sciences")

Abstract

In this study, it was aimed to detect Avian Coronavirus Infectious Bronchitis Virus (IBV), Infectious Laryngotracheitis Virus (ILTV), Avian Metapneumovirus (AMPV) and Avian Reovirus (ARV) agents in broiler and layer flocks by Real-Time PCR (qPCR). For this purpose, tracheal swabs taken from 48 broiler and 45 layer flocks with respiratory symptoms in 15 provinces in Turkey were inoculated into SPF embryonated chicken eggs for virus isolation. The presence of IBV, ILTV, AMPV, and ARV was identified by qPCR. The results revealed that the most common virus in both broiler and layer flocks was IBV with incidence rates of 58.33% and 46.67%, respectively. The incidences of ILTV, AMPV, and ARV in the samples were 22.22%, 13.33%, and 4.44% in layer flocks, and 2.08%, 8.33%, and 20.83% in broiler flocks, respectively. IBV+AMPV coinfection was found in 5 (11.11%) layers and 1 (2.22%) broiler, however, IBV+ARV coinfection was found in 1 (2.08%) layer and 5 (10.42%) broilers. In addition, it was determined that IBV, AMPV, and ARV were found together in 2 broiler flocks (4.17%). Other viruses were not detected in the samples in which ILTV was detected. The sequence of the *S1* gene of selected IBV TR/L45 and TR/B42 isolates showed 98.98% and 99.69% similarity with IS/1494/06 (HM131453), respectively, while TR/L37, TR/L38, and TR/L39 isolates were showed similarity with the 4/91 (KF377577) vaccine strain at a rate of 99.01%, 99.01%, and 98.76%, respectively. As a result of the sequence analysis of ILTV isolates based on *ICP4* and *TK* genes, it was determined that all of them were field strains and low virulence. The available data represent current information on the IBV and ILTV genotypes circulating in poultry flocks in Turkey.

OP[16] Reduction of Chondronecrosis with Osteomyelitis Lameness in Broilers Fed Metal Amino Acid Complexes Using Two Challenge Models

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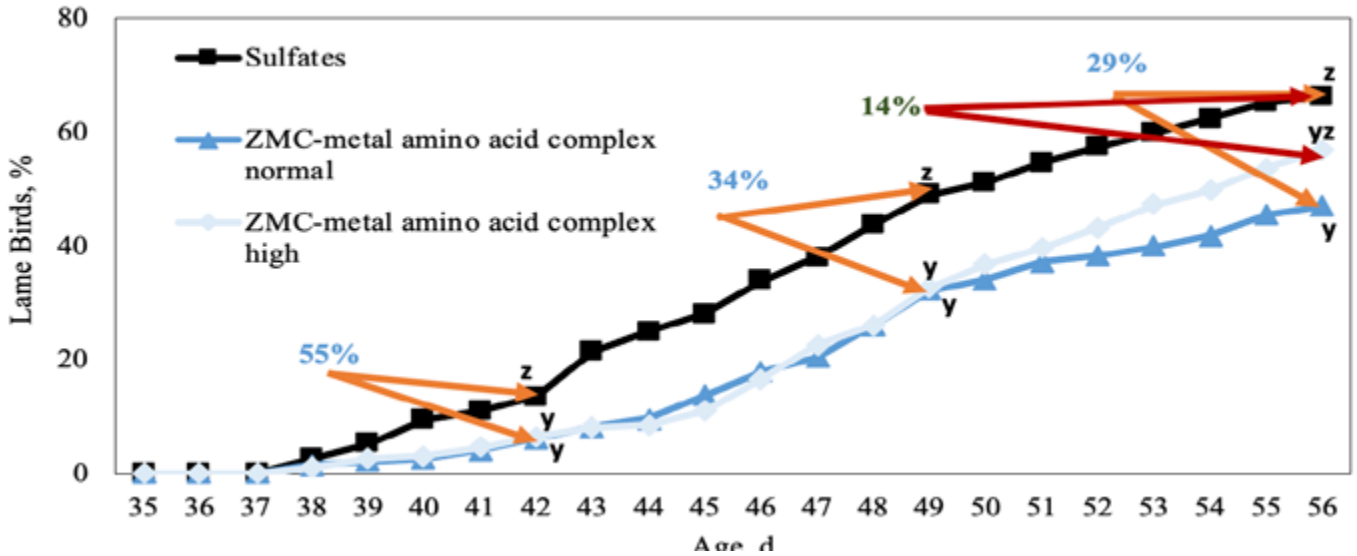
Abstract

The feed additive Zn, Mn and Cu amino acid complex was investigated for the ability to reduce lameness in broilers using 2 alternative models for inducing lameness. The mixture of organic trace minerals was effective in reducing lameness by 20% in the wire flooring model and 25% in the litter flooring model with the bacterial challenge. Lameness in both models is overwhelmingly attributable to bacterial chondronecrosis with osteomyelitis. The reduction in lameness was associated, at least in part, with enhanced intestinal barrier integrity mediated by elevated expression of tight junction proteins and stimulation of bactericidal killing of adherent peripheral blood monocytes obtained from the birds treated with Zn, Mn, and Cu amino acid complex. Lameness is a major animal welfare concern in broiler production. The wire flooring model and litter flooring model with the bacterial challenge are effective models for evaluation of management strategies for mitigating infectious causes of lameness.



Keywords: broiler, lameness, chondronecrosis, *Staphylococcus*, organic trace mineral

Introduction

Lameness is one of the most significant animal welfare issues in the broiler industry, resulting in annual losses of millions of dollars [1,2]. A wire flooring model has been shown to induce a high incidence of lameness in broilers [1,3,4,5,6]. Lameness induced in this system is overwhelmingly bacterial chondronecrosis with osteomyelitis (BCO) of the proximal tibiae and

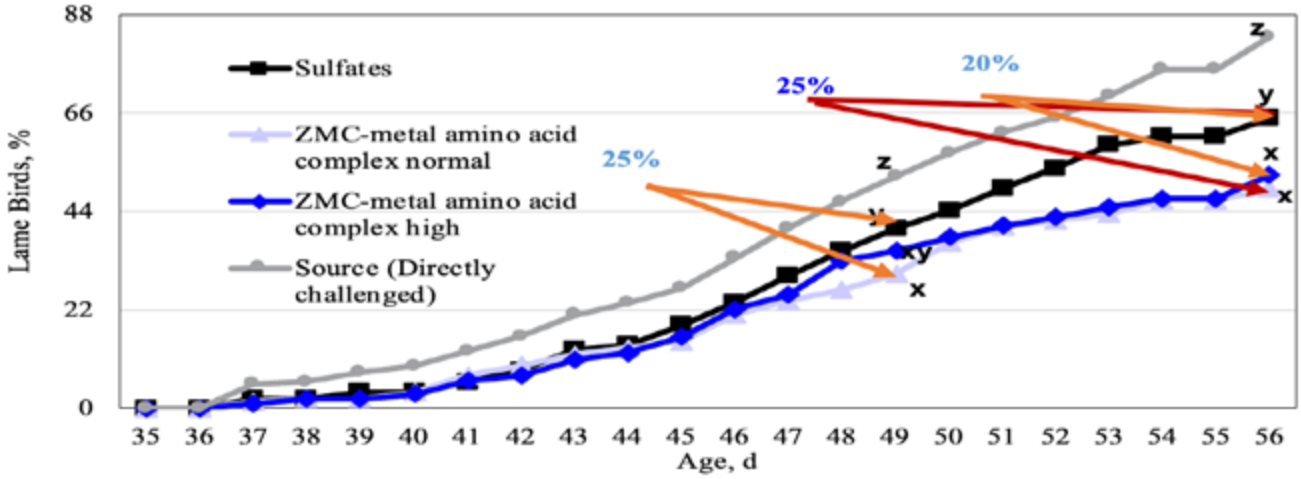
femora [1,3,4,5. The predominant isolates from BCO lesions of birds grown on wire flooring on our research farm are *Staphylococcus agnetis*, and the BCO lameness is sometimes associated with significant bacteremia [7]. The type strain, *S. agnetis* 908, when administered in drinking water can induce high levels of lameness in birds grown on wire or on litter [7,8]. The purpose of this study is to investigation of a commercial combination of zinc, manganese, and copper from metal amino acid complexes using the previously validated BCO lameness model. Organic zinc is reported to enhance epithelial integrity, gut health, and immune function [9,10,11]. Adequate manganese nutrition and copper nutrition are reported to enhance immune function, connective tissue synthesis, and bone development [12,13]. The manganese amino acid complex has shown an improved immune response [14]. The same combination of zinc, manganese, and copper amino acid complexes used in this study has previously shown improvement in bone development in embryos [15]. We therefore hypothesized that supplementation with Zn, Mn, and Cu complexed trace minerals could be efficacious in reducing BCO lameness in our 2 models for inducing BCO lameness.

Materials and Methods:

Lameness Trials: All animal experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee under protocols 18,010 and 18,075. Depending on the model in use, chicks (Cobb-Vantress (Siloam Springs, AR) were placed in 5 X 10 ft. pens on either suspended wire flooring [1,3] or on standard wood shaving litter, at 60 per pen. Feed was standard starter through day 35 and finisher through day 56. In the litter flooring model with the bacterial challenge, source pens were challenged with *S. agnetis* in the drinking water at a concentration of 10^4 colony-forming units per mL. After day 21, the nipple supply was returned to the tap water.

For both wire and litter flooring models, beginning on day 20, all birds were encouraged twice per day to move using standard kitchen brooms. Any bird that was reticent to move was marked with spray paint. Birds that continued to be unwilling or unable to walk were diagnosed as "clinically lame" and euthanized. All birds that died or were diagnosed as clinical lame were recorded by date and pen number. Necropsy for BCO lameness was as described by Wideman, 2016 [1].

For trace mineral dietary supplementation, both inorganic and complex organic sources were used. Inorganic sources consisted of zinc sulfate ($ZnSO_4 H_2O$), with 35.5% of Zn, manganese sulfate ($MnSO_4 H_2O$) with 32.0% of Mn, and copper sulfate ($CuSO_4 H_2O$) with 25.2% of Cu. Availa-ZMC (Zinpro Corporation, Eden Prairie, MN) with 4% Zn, 4% Mn, and 0.7% Cu from amino acid complexes accounted for the organic portion. Details on the supplementation levels are described in Table 1. This supplementation substituted for these minerals in the mineral premix, so no background levels other than those provided by feed ingredients were present. The commercial product was added to the feed before pelleting by the



University of Arkansas Poultry Research feed mill. The mixer and lines were flushed and cleaned between preparations of feed formulations.

Table1: Supplemental levels and sources of minerals added to dietary treatments (ppm).

Treatment	Control: inorganic			ZMC-metal amino acid complex normal[2]			ZMC-metal amino acid complex high[3]		
Mineral	Sulfate[1]	complex	Total	Sulfate[1]	complex	total	Sulfate[1]	complex	total
Zn	100	0	100	40	40	80	40	60	100

Mn	100	0	100	40	40	80	40	60	100
Cu	20.5	0	20.5	10	7	17	10	10.5	20.5

Availa-ZMC (4% Zn, 4% Mn, and 0.7% Cu from the metal amino acid complex) added on top of control diet. This supplementation substituted that for each one of these minerals in the mineral premix.

[1]Zinc sulfate monohydrate ($ZnSO_4$ H_2O), 35.5% Zn; manganese sulfate monohydrate ($MnSO_4$ H_2O), 32.0% Mn; copper sulfate pentahydrate ($CuSO_4$ $5H_2O$), 25.2% Cu.

[2]One thousand milligrams of the commercial product (Availa ZMC) in 1 kg of feed provided additional 40 ppm of Zn, 40 ppm of Mn, and 7 ppm of Cu.

[3]One thousand five hundred milligrams of the commercial product (Availa ZMC) in 1 kg of feed provided additional 60 ppm of Zn, 60 ppm of Mn, and 10.5 ppm of Cu from the amino acid complex

Assay of Intestinal Gene Expression: Sections of the distal jejunum and proximal ileum were collected from 5 apparently healthy birds on day 57 from the control and ZMC-metal amino acid complex treatment groups. One microgram of total RNA was extracted from tissue samples by homogenization using Trizol Reagent (Thermo Fisher Scientific, Rockford, IL) in accordance with the manufacturer's recommendations. RNA concentration, quality, and integrity were assessed by the ratio of absorbance (260/280), and electrophoresis was carried out in 1% agarose gels using a Take 3 microvolume plate and the Synergy HT multimode microplate reader (BioTek, Winooski, VT). RNAs were treated with DNAseI and reverse transcribed using the qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). The cDNA was then amplified by real-time quantitative polymerase chain reaction (PCR) (7500 RealTime PCR system; Applied Biosystems, Foster City, CA) with Power SYBR green Master Mix (Thermo Fisher Scientific, Rockford, IL) in triplicate, with 20 mL per reaction. Oligonucleotide primers specific for chicken were used: occludin (OCLN)—forward 5′-CGCAGATGTCCAGCGGTTA-3 ′ and reverse 5 ′- GTAGGCCTGGCTGCACATG-3′; claudin 1 (CLDN1)—forward 5′-CCCACGTTTTCCCCTGAAA-3′ and reverse 5′-GCCAGCCTCACCAGTGTTG-3′; gap junction protein alpha 1—forward 5′-TGGCAGCACCATCTCCAA-3′ and reverse 5′-GGTGCTCATCGGCGAAGT-3′; and catenin beta 1—forward 5′-TGCCCCACTGCGTGAAC-3′ and reverse 5′-TGCTCTAACCAGCAGCTGAACT-3′. Primers for the reference, housekeeping gene r18S, have been published previously [16,17,18,19]. The cycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, and 58 °C for 1 min with plate read. After PCR, melting curve analysis was applied using the dissociation protocol from the sequence detection system to exclude samples with nonspecific products. PCR products were also confirmed for one specific size band by agarose gel electrophoresis. Negative controls lacked cDNA input as the template for the PCR and were verified for the absence of gel bands. Relative expression of target genes was determined by the $2^{-\Delta\Delta Ct}$ method using r18S as the reference and the control group as the calibrator [20,21].

Phagocytosis Assay: Blood (1 mL) was collected from a wing vein using a vacutainer containing EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ). Monocytes were enriched and cultured using published protocols [22,23]. The medium was RPMI (VWR) with 1× GlutaMax (Life Technologies, Carlsbad, CA) and 10% low-endotoxin fetal bovine serum. After 5 d in culture (37 °C; 5% CO_2), we challenged the adherent cells in triplicate with an approximate multiplicity of infection of 1:1 with *S. agnetis* 908 for 2 d following the published methods [24,25]. Specifically, the bacteria were added to the medium for 2 h; then, the medium was replaced with media supplemented with gentamycin (50 mg/mL) for 6 h to kill noninternalized bacteria. The medium was replaced with an antibiotic-free medium. After 2 d in culture, the adherent cells are lysed by addition of pure water, and a dilution series of the lysate was plated on Luria Broth agar plates for viable bacterial cell counts

Statistical Analyses: Data were compared using either the t-test function in Microsoft Excel or a generalized linear model (GLM) module in R 3.4.2 (https://cran.r-project.org) to produce *P*-values between treatments, as indicated. Gene expression data were analyzed by one-way ANOVA. If ANOVA revealed significant effects, the means were compared using Tukey's multiple range test using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California). Significant difference was accepted at $P \leq 0.05$. Data are expressed as mean ± SEM.

Results

Experiment 1 evaluated whether ZMC-metal amino acid complex could reduce lameness for birds raised on wire flooring to induce lameness. Chicks (1-day-old) were raised to day 56 on wire flooring with no direct administration of a bacterial challenge. There were 4 pens in each treatment group (Details on the supplementation levelsfor each treatment group are described in Table 1). Feed formulations were continuous through day 56, the end of the experiment. Lameness began to

appear in all 3 treatments on day 37, but the trajectory of lameness accumulation was higher for the birds on standard feed (Figure 1). The final cumulative lameness for the control group was 66%, but the ZMC-metal amino acid complex normal treatment group had 47% lameness, and the ZMC-metal amino acid complex high treatment group had 57% lameness. Comparison of the lameness data using the GLM with the individual bird as the experimental unit showed that the ZMC-metal amino acid complex normal treatment was statistically different from the control ($P = 0.0003$) and ZMC-metal amino acid complex high treatments ($P = 0.03$). The control and ZMC-metal amino acid complex high treatment were not statistically different ($P = 0.15$). Pen-to-pen variability for the 3 treatments in experiment 1 reveals a degree of variability in total lame per pen within a treatment (Table 2).

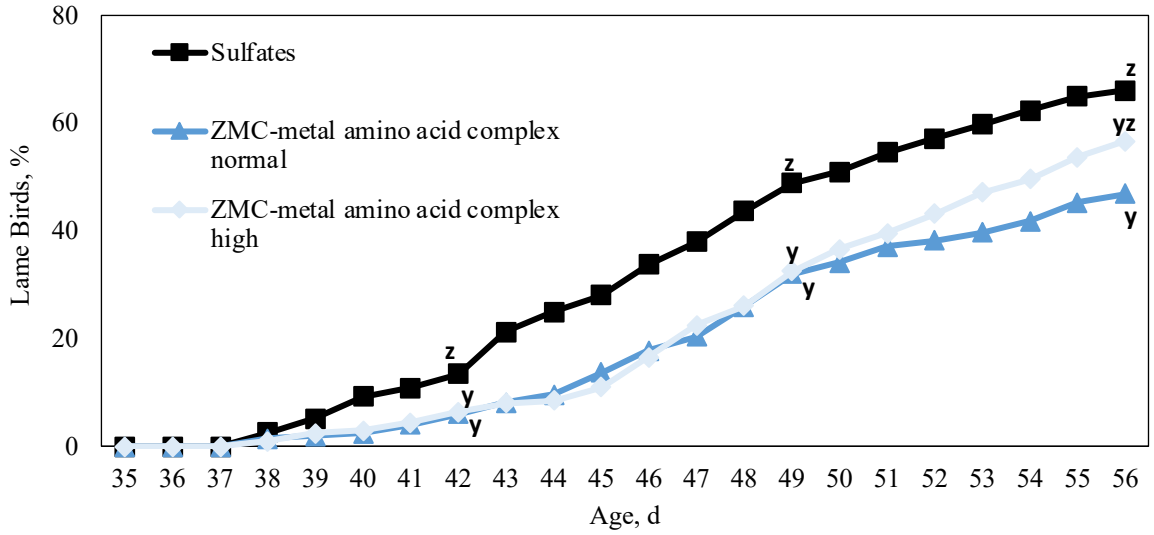


Figure 1. Cumulative lameness for broilers raised on wire flooring treated with control feed or feed supplemented with ZMC-metal amino acid complex. Cumulative percentage of lameness is plotted from day 35 to day 56. Details of the 3 treatments are shown in Table 1. Abbreviation: d, day.

Table 2. Lame by pen and for 3 treatments in experiments 1 and 2.

Experiment	Pen	Count, lame				
		1	2	3	4	Avg[1]
1	Control	36	24	27	40	31.8 ± 3.2[a]
1	ZMC-metal amino acid complex normal	28	25	23	16	23 ± 2.2[b]
1	ZMC-metal amino acid complex high	26	25	43	19	28.3 ± 4.5[b]
2	Control	32	35	32	30	32.3 ± 0.9[a]
2	ZMC-metal amino acid complex normal	22	23	31	26	25.5 ± 1.8[b]
2	ZMC-metal amino acid complex high	26	26	21	28	25.3 ± 1.3[b]

[a,b]Values within an experiment that are significantly different ($P < 0.05$) have different superscripts.
[1]Average (Avg) ± SEM.

Experiment 2 evaluated whether ZMC-metal amino acid complex could reduce lameness in birds raised on litter flooring when a bacterial challenge is imposed. The feed supplementation was the same, but lameness was induced by the transmission of the hypervirulent strain *S. agnetis* 908 from birds challenged with the bacterium in drinking water on day 20 and day 21. There were 3 pens of birds on litter flooring on standard feed that were the source population. These 3 pens were "upwind," relative to the exhaust fans, of the treatment pens (Table 1). There were 4 pens for each of the 3 treatments: control, ZMC-metal amino acid complex normal, and ZMC-metal amino acid complex high, arrayed in a randomized block design and separated by at least 3 m from the source pens. Lameness began to appear on day 36, but lameness accumulation was accentuated in the source population (Figure 3). Accumulation of lameness in the 3 treatment groups lagged behind that for the source population by about 3 to 4 d through day 48, when the cumulative lameness in the control group continued to parallel that for the source population, but the lameness accumulation is reduced for both ZMC-metal amino acid complex treatments. Final percentage of lameness was as follows: source, 83%; control, 65%; ZMC-metal amino acid complex normal, 49%; and

ZMC-metal amino acid complex high, 52%. GLM-based comparisons of the lameness data with the individual bird as the experimental unit showed that the percentage of lameness was statistically higher in the control treatment than in the ZMC-metal amino acid complex normal ($P = 0.002$) and ZMC-metal amino acid complex high ($P = 0.006$) treatments. Pen-to-pen total lame was more uniform in this experiment compared with experiment 1 (Table 2).

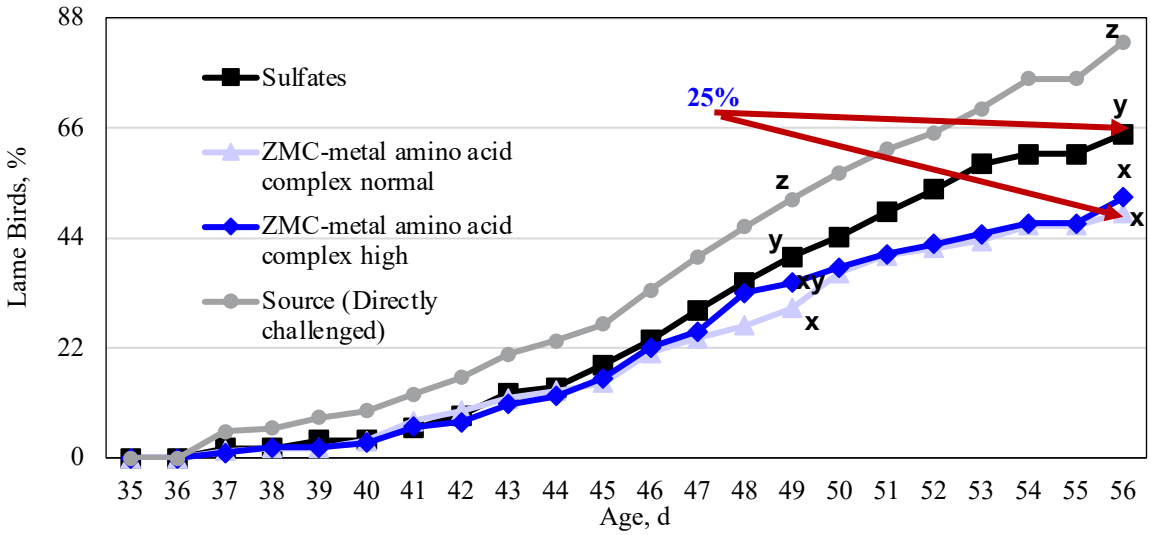


Figure 3. Cumulative lameness for broilers with a bacterial challenge, raised on litter flooring, treated with control feed or feed supplemented with ZMC-metal amino acid complex in experiment 2. Cumulative percentage of lameness is plotted from day 35 to day 56. Details of the control and ZMC-metal amino acid complex treatments are shown in Table 1. The source population was the same as the control population but was challenged with *S. agnetis* 908 at a concentration of 10^4 cfu/mL in drinking water at day 20 and day 21. Abbreviation: d, day

Sections of the distal jejunum and proximal ileum were collected from 5 apparently healthy birds on day 57 from the control and ZMC-metal amino acid complex treatment groups. In terms of important determinants of villus integrity, we examined expression of critical tight junction genes by reverse transcription quantitative PCR. The ileum and jejunum from the samples of the Availa-ZMC high treatment group showed significantly upregulated expression for CLDN1, OCLN, GJA-1, and CTNB1, compared with the control group (Figure 4). Wing vein blood was collected from the same birds examined for intestinal histopathology. Monocytes were enriched, and adherent cells were cultured for 5 d. The cells were then used in phagocytosis assays against *S. agnetis* 908 at an approximate multiplicity of infection of 1:1. Bacterial survival was assessed after 2 d (Table 3). The bactericidal activity was variable between birds within each treatment. The most variation was observed in the birds of the ZMC-metal amino acid complex normal treatment group, in which the adherent cells of bird 3 were highly active in killing *S. agnetis* 908. For the lowest dilution plated, 10^{-2}, there were only 5 colonies from one of the 3 triplicate wells. Therefore, verifying that, bacteria were added, but the bacterial survival was very low within the monocytes from this broiler. The high variability for the 5 birds from the ZMC-metal amino acid complex normal treatment group meant that this treatment group was not statistically different from either the control or ZMC-metal amino acid complex high treatment groups. Bactericidal activity of cells from the birds of the ZMC-metal amino acid complex high treatment group was higher than the activity of cells from the birds of the control treatment group (t test, P = 0.00085).

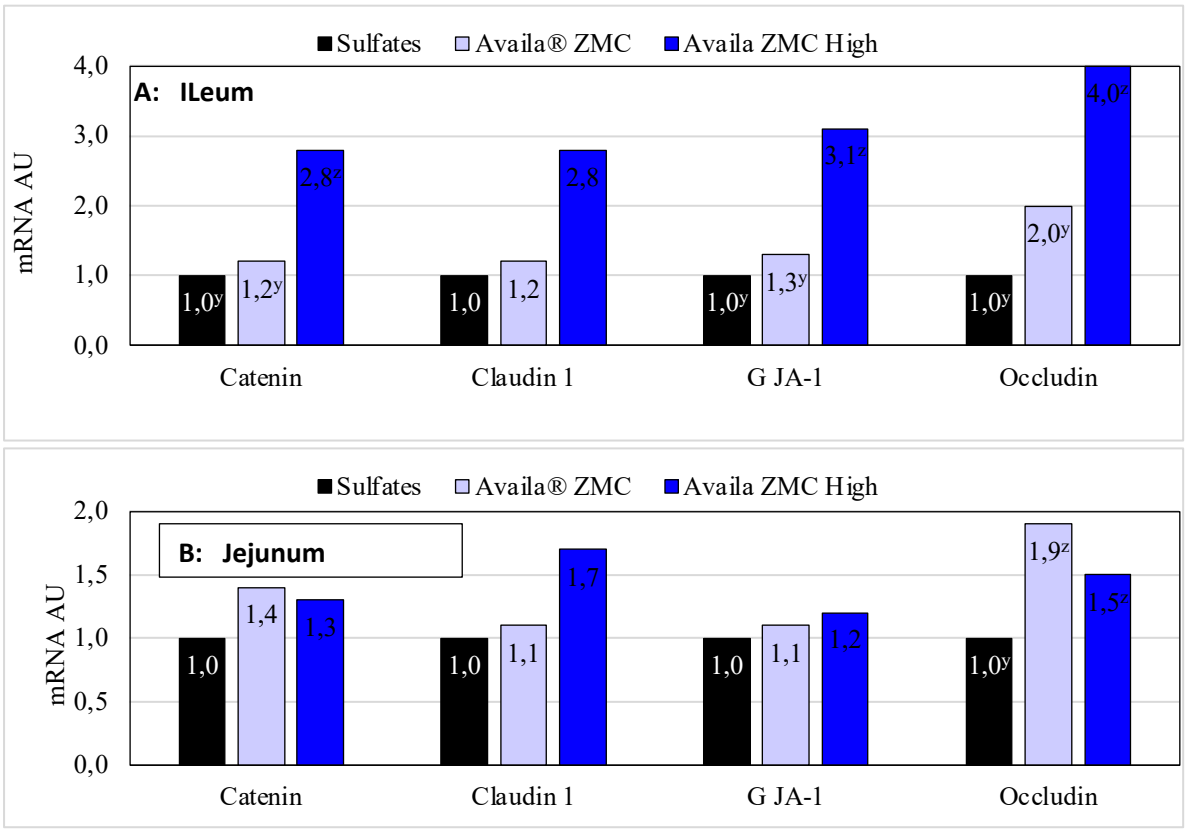


Figure 4. Expression of intestinal barrier integrity–related genes from 3 treatment groups in experiment 2. The relative expression of ileal and jejunal OCLN, CLDN1, GJA-1, and CTNB1 was determined by quantitative PCR and analyzed by the $2^{-\Delta\Delta Ct}$ method using the control group as the calibrator. Data are presented as mean ± SEM (n = 6/group). Different letters indicate significant difference at P <0.05. Abbreviation: PCR, polymerase chain reaction.

Table 3. Bacterial survival in adherent peripheral blood monocytes from 5 birds from the 3 treatments in experiment 2.

Bird	Treatment		
	Control[1]	ZMC-metal amino acid complex normal[1]	ZMC-metal amino acid complex high[1]
1	1.3×10^7	1.6×10^7	5.8×10^6
2	1.7×10^7	1.2×10^7	1.1×10^7
3	1.7×10^7	1.7×10^2	9.9×10^6
4	1.6×10^7	1.1×10^7	7.6×10^6
5	1.2×10^7	2.0×10^7	8.6×10^6
Average[2]	$1.5 \times 10^{7,a}$	$1.2 \times 10^{7,a}$	$8.6 \times 10^{6,b}$
SEM	9.2×10^5	3.0×10^6	8.2×10^5

[1]cfu average from triplicate wells—details of the assay mentioned in Materials and Methods.

[2]Values which are significantly different (P < 0.05) have different superscripts.

Discussion

In our previous research publications, we had reported that *S. agnetis* 908 can induce 80 to 90% lameness when administered to birds raised on wire flooring [7,26]. We also demonstrated that S. agnetis 908 can induce 50 to 80% lameness when administered in drinking water to birds raised on litter flooring [8]. Furthermore, the lameness can be transmitted from the birds challenged with bacteria to unchallenged birds within the same broiler house. The combination of organic zinc, manganese, and copper has been reported to improve poultry health, reduce bacterial pathogen colonization, and reduce femoral head necrosis [27,28]. The results from experiments 1 and 2 confirm that ZMC-metal amino acid complex is effective in reducing lameness in both models for inducing lameness. When birds were raised on wire flooring with no direct bacterial challenge, the mineral supplement reduced lameness by 14 to 29% (Figure 1). In the litter flooring model with the bacterial challenge, the ZMC-metal amino acid complex treatment similarly reduced lameness by 20 to 25% relative to the control treatment (Figure 3). Most importantly, this latter model uses the contagious spread of the infection observed in some broiler operations,

whereas our model uses a hypervirulent bacterial strain; the reduction could be greater against less virulent species/strains. Although the ZMC-metal amino acid complex normal treatment had lower levels of zinc, manganese, and copper than the other 2 treatments, lameness was also reduced compared with the control. This could result if organic trace minerals have higher bioavailability than inorganic sources [9,14,15]. Interestingly, the organic trace mineral upregulated the expression of the genes for tight junction (CLDN1 and OCLN), gap junction (GJA-1), and desmosome (CTNB1) proteins, consistent with improved gut barrier integrity. Although the exact functions of the individual tight junction proteins remain elusive, in avian species, occludin has been reported to be an integral component in tight junction barrier function [29]. Studies conducted in occludin-deficient mice showed gut inflammation and defective epithelial barrier function [30]. Similarly, it has been reported that downregulation of CLDN1 can drastically reduce barrier integrity [31]. Upregulation of these genes is consistent with ZMC-metal amino acid complex (Availa-ZMC) enhancing barrier functions and reducing translocation of bacteria into the blood, a critical first step in the progression of BCO lameness [1,4,26]. Furthermore, the organic trace minerals appear to enhance the bacterial killing activity of adherent peripheral blood monocytes (Table 3). ZMC-metal amino acid complex (Availa-ZMC) has been reported to improve intestinal health, epithelial integrity, and immune function [9,10,11]. The reduction in bacterial lameness in both models could result from either enhanced barrier function or enhanced bactericidal activity of phagocytes, or both. Growth on wire flooring increases translocation of bacteria into the blood relative to growth on litter flooring [26]. The litter flooring model with the bacterial challenge involves noncontact spread of the infection from the source population, and we have speculated on whether the infection is through the pulmonary or gastrointestinal path [8]. The adherent peripheral blood monocyte phagocytosis results suggest that the ZMC-metal amino acid complex reduces lameness in part by enhanced killing of bacteria that translocate into the blood on either type of flooring [26]. The enhanced gene expression data for gut integrity markers argue that both immunity and barrier functions have been enhanced for the ZMC-metal amino acid complex –treated birds raised on litter flooring.

Conclusion

The data reported herein demonstrate that Availa-ZMC can reduce lameness in both the wire flooring model and the litter flooring model with the bacterial challenge. In addition, we observed that ZMC-metal amino acid complex shows a dose dependent enhancement of bacterial killing activity by adherent peripheral blood monocytes cultured from treated birds. These data extend the range of products that can be used to reduce BCO lameness, highlight the importance of organic trace minerals in improving animal wellbeing, and provide further validation of the 2 models we have developed to investigate treatments and management strategies for reducing BCO lameness in broiler operations.

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OP17 Effects of Two Different Microbial Strategies For Broiler Production Under Live Coccidiosis Vaccine Treatment

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Abstract

Coccidiosis remains the major threat in poultry production leading to major economic losses. Anticoccidial drugs (synthetic drugs and ionophores) have been intensively used to control this parasitic disease in broilers. Recent concerns on the emergence of Eimeria strains resistance to some drugs and the raise of the customers demand toward a production with no antibiotic ever have led to find alternative solutions. Live coccidiosis vaccines are increasingly adopted as an alternative prevention to anticoccidials molecules. Vaccination stimulates immunity to prevent coccidiosis, but numerous publications reported the negative impact on zootechnical performances. In that context, this study investigated two new ways to mitigate the negative effect of cocci vaccine on performances throughout two 33-day grow-out trials: 1. Application the first day of life of a competitive exclusion product; 2. Feed supplementation with a feed ingredient based on yeast polysaccharides all along the production cycle. Both trials confirmed the impact of live coccidiosis vaccine on performances. In the first trial, conducted under a favorable sanitary context, early alteration of performances by the vaccination were depicted (at day 25). Both BW, ADG and FCR from the competitive exclusion group were not significantly different from the non-vaccinated at day 33 showing that this microbial solution alleviates the detrimental effect of the vaccine on performances. In the second trial, performed under more challenging conditions, the effect of vaccination on performance was detected earlier (from day 10). The beneficial effect of the yeast polysaccharides supplementation was mostly detected on the FCR.

Introduction

A healthy gut microbiota is essential for nutritional efficiency and to maintain natural defenses. The chick's microbiota establishment takes several days after hatching, a time when the birds are particularly sensitive to external challenges.

In poultry production, the pressure to reduce reliance on anticoccidial molecules is increasing all around the world. In the US ionophores are already excluded for Non Antibiotic Ever (NAE) productions. The rise of microbial resistance to chemical molecules leads to find alternative solution. In this context cocci vaccine is spreading in poultry facilities but not without potential deleterious effects on growth performances.

The main objective of this investigation was to assess the impact of live coccidiosis vaccination on zootechnical performances used alone or in combination with two microbial strategies: a competitive exclusion product and yeast polysaccharides. Evaluation criteria were based on broiler performance and oocysts excretion in feces.

Materials and method

Animals : In two separate trials, 150 & 135 day-old mixed sex ROSS 308 broilers were involved respectively to compare 3 treatments: negative control (NC), vaccine group and vaccine group with a microbial solution: a competitive exclusion product (AviGuard® - Lallemand SAS) and yeast polysaccharides (Optiwall® - Lallemand SAS). Each group was made of 3 floor-pens (1m^2 each pen) of 15 chicks, except the NC with 4 replicates in the first trial. Upon arrival, all chicks were weighed and randomly allocated to one pen until an even average pen weight was reached across pens. They had immediately access to water. The lightest (<33g) and the heaviest birds (>49g) were not selected at grouping with the objective to minimize standard deviation.

Coccidiosis vaccine : The coccidiosis vaccine used in the trial was EVALON® (HIPRA). Different batches of vaccine were used in trial 1 and 2. The vaccine contains a mix of 5 different Eimeria strains*: E. acervulina, E. brunetti, E. maxima, E. necatrix, E. tenella*. The manufacturer mentions that using this vaccine at 10 times the recommended dosage can induce temporary reduction of daily weight gain in the first week, without any consequence on final outcomes. The dosage used in the current trial was set at 5x the recommended application rate in order to get an accurate count of oocysts in droppings (Mathis et al., 2018). The dose was set to fit into a 1mL pipette as a standardized volume per bird. We adjusted the total

volume (water + adjuvant + vaccine) indicated for 1000 birds (280 mL) to the volume applied to 200 birds, i.e. 5x the application rate. The vaccination was performed right after the allocation of the birds in their respective pens by individual gavage and prior feed access.

Diets : Starter crumbles diet without anticoccidials was formulated and offered throughout the trial from 1 to 33 days. Main feed ingredients were maize (44%), soybean meal (23.5%), wheat (13.8%), sorghum (7%) and sunflower (5%), for a target metabolic energy of 26.97 MJ/kg (AME broiler).

Treatments : For the first trial, once the 3 pens of vaccine groups were inoculated (i.e. 45 birds), the remaining volume of vaccine was completed with the corresponding amount of a competitive exclusion microbiota and stirred until full mixing of the powder. The last 3 pens were then inoculated with vaccine and the competitive exclusion mixed preparation at 1 mL per bird too. Control birds also received 1 ml of mineral water.

For the second trial, the yeast polysaccharides product was supplemented at the dose of 1kg/ton of feed until d10 and at the dose of 0.5kg/ton feed from d11 to d33. The product was mixed into the crumbled feed directly on the farm.

Measurements : Chicks were individually weighted at d1, d5 (trial 1 only), d10, d25 and d33. Feed delivery and refusal were calculated for each pen on the day of the weighing. Average daily gain (ADG) and feed conversion ratio (FCR) were determined with the number of live birds and the number of days-birds. Oocysts were counted in feces at d10 & d25, as a proposed marker of vaccinal oocysts recycling (Repérante *et al*., 2022).

Statistics : Treatment groups comparison was performed using a non-parametric Kruskal-Wallis test (SPSS Statistics 27.0, IBM). Pairwise comparison was established as an indication of the dietary effect even if a trend was depicted.

Results

For both trials, the live oral coccidiosis vaccination induced a significant reduction in body weight at d33 of age (-6.6% and -9.0% respectively for trial 1 and 2), and even from d25 for the first trial (-8.8%) and from d10 in the second trial (-9.1%) as shown in Tables 1 and 2. The average daily gain from d1 to d33 was also significantly altered (-7.5% and – 9.2% respectively). Feed intake was not influenced by any treatment. As a consequence, FCR was negatively impacted (+12.3% and +5.0% respectively) albeit only numerically in trial 2. This negative effect of vaccination on FCR was detected from d25 in the first trial (d10-25: +19.1%; d25-33: +5.2%).

Results from the first trial (Table 1) indicated that vaccinated birds receiving an inoculation with the competitive exclusion at d1 had significantly better performances (body weight, live average daily gain, FCR) than control vaccinated birds. Non-significant differences were found between non vaccinated birds and vaccinated birds inoculated with the competitive exclusion product except at d25 where performances were intermediate between non vaccinated and competitive exclusion vaccinated group.

Results from Table 2 indicated that all vaccinated birds were impacted by the vaccination but numerically to a lesser extent for the supplemented group. Statistical differences were detected for the FCR with a better FCR from day 25 onwards in the supplemented group.

Table 1. Zootechnical performance per period for the 3 groups (a,b,c: P ≤ 0.1). Means and (standard deviation) for the competitive exclusion inoculation strategy (trial 1).

Mean (SD)	Day or Period	CONTROL	CONTROL + VACCINE	Competitive exclusion + VACCINE	P-value
Body weight (g)	1	39.2 (0.10)	39.3 (0.05)	39.4 (0.17)	0.260
	5	98.3 (2.5)	98.6 (1.2)	96.5 (1.7)	0.397
	10	234.4 (2.4)	232.0 (2.7)	230.6 (1.7)	0.153
	25	1221.6 (17.2) a	1114.4 (19.1) b	1157.9 (16.7) c	<0.001
	33	1891.5 (57.2) a	1767.4 (33.4) b	1849.4 (32.7) ab	0.025
Average daily gain (g/bird/day)	1_5	11.8 (0.5)	11.9 (0.2)	11.4 (0.3)	0.361
	5_10	27.0 (0.6)	26.5 (0.3)	26.8 (0.3)	0.393
	10_25	65.8 (1.2) a	58.8 (1.5) b	61.8 (1.1) c	0.001
	25_33	83.7 (5.2)	81.6 (2.1)	86.4 (2.1)	0.344
	1_33	54.9 (2.0) a	50.8 (1.0) b	53.1 (1.0) a	0.029
Individual daily feed intake (g/animal/day)	1_5	10.9 (0.7)	12.0 (2.3)	10.8 (0.5)	0.469
	5_10	36.1 (1.9)	38.5 (1.8)	35.9 (0.6)	0.145
	10_25	92.5 (10.8)	98.4 (7.6)	91.9 (1.4)	0.569
	25_33	147.7 (8.0)	151.5 (2.0)	154.8 (3.7)	0.328
	1_33	82.9 (4.8)	86.2 (3.4)	83.3 (1.5)	0.500
FCR	1_5	0.92 (0.019)	1.01 (0.168)	0.95 (0.036)	0.471
	5_10	1.34 (0.090)	1.45 (0.063)	1.34 (0.014)	0.126
	10_25	1.41 (0.170) a	1.68 (0.149) b	1.49 (0.006) ab	0.095
	25_33	1.77 (0.031) a	1.86 (0.029) b	1.79 (0.021) a	0.010
	1_33	1.51 (0.100) a	1.70 (0.089) b	1.57 (0.005) a	0.052

Table 2. Zootechnical performance per period for the 3 groups (a,b,c: P ≤ 0.1). Means and (standard deviation) for the prebiotic in-feed strategy (trial 2).

Mean (SD)	Day or Period	CONTROL	CONTROL + VACCINE	Yeast polysaccharides + VACCINE	P value
Body weight (g)	1	39.1 (0.1)	38.8 (0.1)	39.1 (0.2)	1.000
	10	212.3 (7.5) a	194.0 (5.3) b	193.0 (6.1) b	*0.066*
	25	961.8 (2.2) a	858.4 (28.4) b	878.4 (29.3) b	*0.051*
	33	1598.2 (19.7) a	1455.3 (62.7) b	1477.5 (22.8) b	*0.061*
Average daily gain (g/bird/day)	1_10	17.3 (0.7) a	15.5 (0.5) b	15.4 (0.6) b	*0.066*
	10_25	50.0 (0.6) a	44.3 (1.7) b	45.7 (2.1)b	*0.051*
	25_33	79.6 (2.7)	74.6 (5.1)	74.9 (0.8)	*0.099*
	1_33	47.3 0.6) a	42.9 (1.9) b	43.6 (0.7)b	*0.061*
Individual daily feed intake (g/animal/day)	1_10	21.7 (2.7)	20.7 (3.1)	21.9 (1.7)	0.661
	10_25	92.5 (18.6)	93.6 (1.8)	78.4 (3.0)	0.301
	25_33	132.3 (6.4)	132.1 (6.2)	124.3 (3.0)	0.184
	1_33	75.7 (18.3)	80.8 (3.1)	72.2 (2.4)	0.301
FCR	1_10	1.28 (0.14)	1.33 (0.16)	1.45 (0.13)	0.258
	10_25	2.11 (0.08) a	2.11 (0.05) a	1.72 (0.02) b	*0.066*
	25_33	1.66 (0.03) A	1.77 (0.04) B	1.66 (0.06) A	*0.066*
	1_33	1.79 (0.07) a	1.88 (0.01) a	1.66 (0.03) b	0.027

Concerning vaccinated birds, oocyst shedding was identical between groups on d10 and d25, suggesting that a normal cycling of the vaccine oocysts had occurred. For the first trial, the average of oocysts per gram of feces (OPG) was 2 617 OPG at d10 and 767 OPG at d25. For the second trial, similar values were obtained with an average of 5 828 OPG at d10 and 20 933 OPG at d25, indicating that birds were still secreting a high quantity of parasites in the environment. No oocysts were found in the control non vaccinated pens for the first trial, while 2 917 OPG were detected in average at d25 in the control non vaccinated in the second trial.

Discussion

Our results may suggest that coccidiosis vaccination effect on performances depends on the quality of the flock: in a context of optimal performances, the biggest impact was measured during the grower phase. Then, the difference between the vaccinated groups and non-vaccinated group seems to attenuate in the finisher phase, suggesting a potential compensatory growth. The standard broiler production cycle is too short to get the time to recover performances. In contrast with a batch that under-performs (trial 2), where the effect of the vaccine on performances was detected earlier (from day 10 onwards).

Despite the same average body weight at the arrival at the farm at d1, growth curves and performances were depleted in the second trial. Various explanations can be suggested: 1. Origin of the hatchery: chicks from the second trial were fastened during a longer period (12 hours) due to a longer distance between the hatchery and the farm; 2. Chicks quality: despite having removed the smallest chicks, the heterogeneity at the farm arrival was higher in the second trial potentially impacting the heterogeneity of the immune response to the vaccine; 3. Vaccine quality: two different batches of vaccine were used for the trials and as coccidiosis vaccines have a short shelf life we cannot exclude a difference in potency between the two batches of vaccine even if both were stored under the recommended conditions and used before expiry dates. Soutter *et al*. (2020) highlighted this limitation in a recent review including the vaccines used in our study.

The microbial based strategies tested in this study contributed to alleviate vaccination side effects on performances. Both products are based on a well-established concept aiming at reinforcing the immune status of the birds, especially through a faster mature and balanced microbiota establishment (Meijerink *et al*., 2020; Dame-Korevaar *et al*., 2020, Methner et Rösler, 2020). The interest to combine competitive exclusion solution and live vaccines is not new. Several authors previously investigated the interest of reinforcing the effect of live Salmonella vaccine in newly hatched chickens (Braukmann *et al*., 2016; Methner *et al*., 2017) and conclude to the effectiveness of the competition exclusion product. Explanation relies on a synergistic mode of action: live vaccine triggers the innate immune system and the competitive exclusion solution early shapes the gut microbiota, thus creating a favorable physiological environment for beneficial bacteria and reducing the possibility of opportunistic bacteria to develop. Another explanation, which could also be applied to the yeast polysaccharides supplementation, is linked to the modulation of inflammation in the gut, which would minimize the nutrients diversion towards an inflammatory response, to the benefit of birds' performance.

Conclusion

This study presents two microbial based strategies to compensate the previously reported negative effects of attenuated live cocci vaccines on zootechnical performances. Both trials confirmed a negative impact of vaccination on performances and partial (on FCR only) to complete (on FCR and growth performance) restoration of performances were obtained with the two strategies. The level of oocysts excretion in feces remains at the same level in both vaccinated groups, indicating that the microbial-based strategies didn't alter the mode of action of the live vaccine. From these results, it can be concluded that the two strategies are compatible with coccidiosis vaccine and could turn to be viable solutions to alleviate the negative effect of coccidiosis vaccine on performances. Further studies involving a challenge model with a Eimeria contamination and also larger scale studies with more birds would contribute to confirm the potential of the tested additives.

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OP[18] The Effect of Radio Frequency Thawing On Marination Yield and Marine Product Quality of Chicken Breast Meat

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Abstract

In the current study, the breast meat, which was thawed by radio frequency (RF, 10 kW, 27.12 MHz) and two conventional methods (thawing at 22±0.5**°C** or **4±0.1°C),** was marinated and the effect of marination process was examined on undesirable quality changes of freeze-thawing process in meat. Marination increased the water holding capacity and cooking yield, resulted in softer meat texture in all groups and improved superficial color properties in meat thawed at 4°C. Moreover, findings have been proved that softer meat with lower cooking loss and higher water holding capacity could be obtained as a result of improvement effect of marination process on functional properties of Breast meat thawed by RF.

Keywords: Radio frequency, thawing, breast meat, marination, sodium tripolyphosphate, water holding capacity, hardness, cooking loss

OP19 Use of Carrot Juice For Coloring Chicken Meat Cubes With Vacuum Impregnation Technique

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Abstract

In this study, it is aimed to color the chicken breast meat cubes with carrot juice using the vacuum impregnation (VI) technique, and then to dry them into a healthy snack form. Firstly, carrot juices in different concentrations (1, 2 and 4°Bx) were impregnated into meat cubes for 2 hours at 25°C, and then the colored cubes were dried at 60°C. The pH values of the carrot juices used in the study were determined in the range of 6.26-6.37. The moisture content and pH values of the cubes colored with carrot juice were determined in the range of 30.41-32.29% and 5.90-5.93, respectively. When the carrot juice concentration increased from 1°Bx to 4°Bx, the cubes had lower L^* and higher a^* and b^* values. In addition, as the concentration increased, the amount of β-carotene in the colored meat cubes increased from 21.05 **µg**/g to 83.29 **µg**/g. With this study, it was concluded that orange colored functional chicken meat cubes can be produced using vacuum impregnation technique.

Keywords: Carrot juice, quality, dried chicken meat, vacuum impregnation

IS[08] Principles and Implementation of Aviagen's Nutrition Specifications For Broiler Breeders

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Abstract

In this paper the key principles and points of implementation of Aviagen's nutrition specifications for broiler breeders are discussed. The development of the specifications starts with collecting information on the requirements; for this, models (equations) to predict energy and amino acid requirements are used. Following this, results from the models have to be validated in experimental and field trials. The last step is to produce the nutrition specifications based on the established requirements. The two key points of implementation of the nutrition specifications are: Controlling digestible Lysine (dLys) intake by ensuring formulation avoids any excess, this is essential to prevent over-fleshed (heavy) birds and obtain uniform flocks; and diluting Grower and Pre-breeder diets, to support uniformity, prevent undesirable flock behavior and enhance digestion and nutrient utilization.

Introduction

Primary breeding companies work to ensure good technical results for their customer base, which contribute to business profitability. To achieve these objectives, reproductive fitness from balanced genetic selection is essential; in addition, correct management, health status and nutrition are necessary to maximize the reproductive genetic potential of the broiler breeders. The correct nutrition strategy is essential to ensure the correct deliver of energy and nutrients required throughout the different age phases of the birds; in addition, good quality control and feed production processes, and accurate formulation are required. Nowadays, body weight (BW) management is essential in broiler breeders to achieve the correct growth rate; in the rearing period, following the recommended BW profiles helps obtain uniform flocks; this reduces light birds going through under consumption periods and risking leg health, feathering and behavioral issues.

Principles of the nutrition specifications for broiler breeders

The first step in producing broiler breeder specifications is determining the energy and amino acid requirements of the birds; these requirements are estimated using models (equations) of prediction. Models to estimate energy and amino acid requirements take into account maintenance, growth and egg mass. Aviagen over the years has developed and fine-tuned proprietorial prediction models, following a similar logic to those published in the literature, such as Hurwitz and Bornstein (1973), and Leveille and Fisher (1958, 1959, 1960a,b).

Once the energy level of the diet is calculated, the level of dietary amino acids is based on the daily requirements as determined by the amino acid model. For example, if the birds need 634 mg/bird/day of methionine and a feed containing 2800 kcal ME/kg is being fed, to achieve the required 468 kcal/bird/day, the feed intake will be 167 g/bird/day at peak production. To achieve the required methionine intake, the diet will therefore have to be formulated to contain a minimum of 0.38% methionine. This process is repeated for each individual amino acid until all are expressed in dietary density.

The second and third steps are to validate results from modeling in experimental and field trials respectively; in particular amino acid levels. The objective is to confirm the accuracy of the energy and amino acid requirements, in order to produce specifications allowing nutritionists to confidently formulate diets. Formulation based on the nutrition specifications, together with correct feeding management, is key in the rearing period if the standard BW profiles are to be successfully followed, and importantly promote correct body condition (fat deposition over breast meat) leading to biologically uniform flocks. In the laying period, formulas meeting the specifications provide the required energy and nutrients to sustain peak and persistency of production, hatchability, and chick quality. However, in practice, it may be necessary to adjust precisely nutrient delivery, up or down in response to performance results, health and management challenges, or environmental conditions such as temperature or humidity; all these factors should be considered as they influence the bird's requirements.

Implementation of the nutrition specifications for broiler breeders

Accurate formulation following Aviagen's nutrition specifications is essential to meet the requirements of the Aviagen broiler breeder. Energy, crude protein, essential amino acids, minerals, trace minerals and vitamins play important metabolic roles, which contribute to physiological development and sustain production. This section of the document focuses on digestible lysine intake control and the dilution of grower and pre-breeder diets; these are two key points regarding the implementation of the specifications.

Digestible lysine intake control

Broiler performance improvements are largely the result of selection based on balanced protein response, as the objective is lean meat growth. As a result, broiler breeders are highly responsive to digestible lysine (dLys); this has to be managed if optimal results are to be achieved.

Higher dLys intake than recommended will lead to loss of BW control (over-fleshed birds), this in turn risks over-restriction to bring BW down to the standard, and in consequence poor uniformity. Additionally, it is critical to establish a strict control on energy : dLys ratio; otherwise, the propensity of the bird to utilize excess of dLys for breast deposition will promote lean body weight gain. This may be attributable to increased maintenance requirement (ME) for over breasted birds, caused by excessive dLys / other amino acids intake, leading to low abdominal fat reserves at point of lay; unless feed intake is increased even higher which turns out to be inviable due to the need to follow a pre-established BW profile.

Therefore, it is very important to formulate broiler breeder diets meeting the published dLys levels avoiding any excess. In diets low in dLys (Gower, Pre-breeder, and Rooster diets), to formulate the recommended dLys values while at the same time ensuring a good pool of non-essential amino acids (critical for the development and maintenance of feather cover), driven by crude protein (CP), may require the inclusion of vegetal protein concentrates low in Lysine, for example corn gluten meal.

Diluted diets to broiler breeder pullets

Reducing the concentration of the broiler breeder pullet diets by including low energy and nutrient raw materials, diluents, is a nutritional strategy to increase feed allocation. Due to low daily feed volumes, Grower and Pre-breeder diets are usually diluted; raw material sources of insoluble fiber are the most common diluents used to dilute pullet diets.

Increasing feed allocation is an important tool to facilitate homogeneous feed distribution and lengthen feed clean-up time. This will result in more uniform access to the ration and therefore will prevent aggressive birds from out-competing timid ones. Accordingly, feed dilution contributes to reducing BW variation, to produce uniform flocks. In addition to helping with flock uniformity, increasing feed allocation promotes the feeling of satiety, which in turn may help to avoid abnormal behaviors. Asensio *et al.* (2020) studied the effects of fibrous diluted diets, fed to broiler breeder pullets, on grasping feather pecking and non-food object pecking. At 22 weeks of age, compared to the standard-control diets, pullets fed the diluted diets showed a significant reduction of these abnormal behaviors.

Apart from the advantages explained previously of diluting pullet diets, fiber diluted diets have the following benefits for the digestion process:

- If fibrous ingredients are consumed, their particles are retained for a longer time in the crop compared to other feed particles (Vergara *et al.,* 1989); where considerable moisturization takes place. Therefore, any exogenous enzymes and other components that are activated by moisture will potentially be able to exert their effect in the crop. The time needed for soaking may be a critical factor in determining the efficacy of an exogenous enzyme, provided that the crop is indeed a major site of enzyme activity (Svihus, 2014). Consequently, longer retention time in the crop, mediated by fibrous ingredients, will aid enzyme activity.

- Feed particles mixed with the proventriculus secretions arrive at the gizzard. Several published studies report beneficial effects of fiber on gizzard function, mostly due to mechanical stimulation (Hetland *et al.,* 2005; Hetland and Svihus, 2007, Sacranie *et al.,* 2012). The high lignin content of most insoluble fiber sources leads to a longer retention of the feed in the gizzard, improving its muscular development and thus its function (Hetland and Svihus, 2001; Hetland *et al.,* 2003; Jiménez-Moreno *et al.,* 2010).

- Once feed particle size is reduced by the gizzard, the digesta passes to the small intestine where there are intensive gastroduodenal refluxes to compensate for its small dimensions and fast transit rate. Fiber inclusion in the diet is thought to improve the number and intensity of these refluxes (Hetland *et al.,* 2003). Likewise, fiber fractions enter into the

caecum by antiperistaltic movements; in this organ a large bacterial community breaks down indigestible plant material; in fact, these microorganisms ferment part of the fiber, obtaining short chain fatty acids that reduce caecum pH and may be used as a preferential source of energy by the colonocytes.

Conclusions

Models are used to calculate the energy and amino acid requirements of the broiler breeders; after validating the results in experimental and field trials, they are used to produce the nutrition specifications. Formulation based on the specifications is key in the rearing period to follow the standard BW profiles, promote correct body condition and obtain uniform flocks; in the laying period, formulas meeting the specifications sustain peak and persistency of production, hatchability, and chick quality.

Broiler breeder diets have to meet the dLys levels in the nutrition specifications avoiding any excess. Higher dLys intake than recommended leads to loss of BW control (over-fleshed birds), this in turn risks over-restriction and in consequence poor uniformity. Due to low feed allocation during grower and pre-breeder periods, these diets are susceptible to dilution. This nutritional strategy increases daily feed intake, thus promoting better flock uniformity, and may help reduce undesirable flock behavior and enhance the digestion process.

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OP20 Impact of Lignocellulose Inclusion in Diets on Selected Productive and Reproductive Parameters in Poultry

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Abstract

This paper reviews the impact of dietary lignocellulose (Arbocel®) on litter quality, performance and carcass characteristics of broilers as well as on nutrients digestibility in roosters and performance, egg grade, and hatchability rate of broiler breeder eggs. Compared to control birds, litter moisture content of broilers fed rations with 0.8% of Arbocel® instead of wheat bran (WB), was reduced by 10% ($p<0.01$). Dietary Arbocel® did not affect the birds' average market live weight; however, it improved their protein digestibility which reflected positively on their ready to cook carcass yield (69.5%) and reduced their abdominal fat (1.11%) compared to those of the control birds (68.4 and 1.23%, respectively). Results also showed that 0.8% Arbocel® supplemented broiler diets enhanced protein digestibility of 55 week old Hy-line roosters by 6% ($P<0.05$) and improved apparent and true amino acid digestibility when compared to control diets containing no or 0.8% WB. In 33-week old broiler breeders and over the 6-month trial, the 0.8% Arbocel® diet, compared to control, decreased percent infertility and increased average egg hatchability by 4.07% ($P<0.05$). In conclusion, Arbocel® fed group had 3.8 more hatching eggs per housed hen compared to control birds with 5.7 more saleable chicks during the 6 month trial; a sizeable profit to the breeder grower.

Introduction

High levels of soluble polysaccharides in poultry diets have been associated with poor digestibility of feed constituents and nutrients uptake, and may cause sticky droppings and wet litter problems (Barbour et al., 2006; Farran et al., 2010). However, insoluble polysaccharides, such as lignocellulose, hold positive effects regarding the digestion of nutrients (Hetland et al., 2005). They affect the movement of digesta in the intestine and may enhance some enzymes to have good access to their substrates by forming a spongy structure in the digesta (Choct, 2001). In addition, insoluble fibers have an effect on the villi height in GI tract, the issue that will make absorption and retention of nutrients better and resulting in better carcass weight and carcass yield. Weight gain, feed conversion and growth were reported to be increased as well due to insoluble fiber supplementation. Regarding breast meat and carcass percent, a positive significant result was also recorded (Awad et al., 2008; Dohms, 2006). The present paper reviews the impact of inclusion of lignocellulose (Arbocel®) in poultry diets at a rate of 0.8% on performance, carcass characteristics, litter quality, nutrients digestibility and egg hatchability as collated from four trials conducted in our laboratory at the American University of Beirut. These works were carried out on broilers, broiler breeders and mature roosters in environmentally controlled poultry houses.

Materials and Methods

Trial 1: A total of 1200 one-day-old male chicks of Ross 308 strain were placed in floor pens of 100 each. All pens had a bedding of wood shavings with a thickness of 8 cm. Starter and Finisher diets were used in this study (Table 1). The experimental group received 0.8% Arbocel® as a substitute for 0.8% wheat bran for the control diet. The weight of the animals in each pen, the feed conversion (FC) and litter moisture levels were recorded on the 21st and 38th days. Carcass weights (ready to cook) and the weight of abdominal fat of 10 chickens/pen, i.e. 60 chickens per group, were recorded on the 38th day.

Table 1. Composition of the starter and finisher diets

Ingredients (%)	Starter (1-21 d)		Finisher (22-38 d)	
	Control	Experimental	Control	Experimental
Corn	52.648	52.042	61.172	60.566
Soybean Meal (48% CP)	37.732	38.090	30.017	30.375
Arbocel®	-	0.800	-	0.800
Wheat bran	0.800	-	0.800	-
Sunflower oil	4.744	5.000	3.838	4.085
Salt	0.445	0.446	0.443	0.444
Limestone	1.176	1.170	1.172	1.166
Dicalcium Phosphate	1.792	1.803	1.851	1.862
DL- Methionine	0.285	0.285	0.25	0.251
L-Lysine HCL	0.016	0.010	0.094	0.088
Vitamin & Mineral Mix	0.300	0.300	0.300	0.300
Coccidiostat	0.0625	0.0625	0.0625	0.0625
Calculated Analysis				
ME (Kcal/Kg)	3200	3200	3200	3200
CP (%)	23.0	23.0	20.0	20.0
TSA (%)	1.00	1.00	0.88	0.88
Lysine (%)	1.25	1.25	1.10	1.10

Trial 2: Apparent Digestibility measurement: In parallel with Trial 1, twenty four one-week-old Ross 308 broiler males were selected to determine the protein and lipid digestibility coefficients. There were 2 treatments (0.8% Arbocel® and Control), 3 replicates/treatment and 4 birds/replicate. The method described by Fuenté et al. (1998) was used as a reference to determine the apparent digestibility. At 14 days of age, the chickens were deprived of feed for 16 hours while having water ad libitum. Then, the feed was reintroduced for a period of 4 days followed by 16 hours of fasting to empty the digestive tract of as much of its contents as possible. During this period total excrement from each group was collected and dried. Feed and faeces were analyzed for moisture, fat and protein content (AOAC, 1998). This made it possible to determine the coefficients of apparent digestibility of fat and protein. Data were analyzed by ANOVA (SAS, 1992).

Trial 3: Rooster Digestibility Trial. A total of 36 mature dubbed Hy-Line W36 roosters (aged 55 weeks) were randomly divided into 4 groups of 9 birds each. One group was given the control diet, another group given the Control plus 0.8% wheat bran (WB) and one group given the Control plus 0.8% Arbocel® (Table 2). Forty-eight hours prior to precision feeding, feed was removed and each bird was intubated with 40 ml of aqueous glucose solution (50% w/v) at 8 and 24 hours after the start of fasting (McNab and Blair, 1988). The diets were ground to 2 mm particle size and 9 birds in each of the 3 groups were individually intubated 30 g of each diet. The remaining 9 birds in the fourth group, were individually fed 30 g glucose powder to estimate endogenous and metabolic energy and amino acid losses. Total excreta of each rooster were collected during the whole 48 h post-feeding period, dried at 105^0 and ground. Dried feed and feces samples were analyzed for gross energy, proximate chemical composition (AOAC, 1998) and amino acid concentration (HPLC). The apparent and true amino acid digestibility of the test diets were calculated using Likuski and Dorrell's method (1978). Data were analyzed as one way ANOVA and means were compared by Tukey's method at $P<0.05$ (SAS, 1992).

Trial 4: Broiler breeder trial. A total of 26,000 broiler breeder layers of Ross 308, in their post-peak period (33 weeks of age) and 2,600 roosters were raised in 6 adjacent layer houses standing in parallel to each other. A complete randomized design was used with 2 treatments and 3 replicates (houses)/treatment with an average of 4,330 hens and 433 roosters/house. The layer rations were either mixed with 0.8% wheat bran or 0.8% Arbocel® (Table 2). At the start of the trial, birds were 33 weeks of age and data were collected over a 6 month period. Egg production was recorded daily and eggs were graded into different categories. Each month, a total of 864 eggs were sampled from each house. Infertility was determined in percentages of total eggs set after candling randomly selected eggs on the 10th day of incubation. Day-old chicks were counted, graded and percent hatchability was computed. All data pertaining to egg production, infertility, and hatchability along with mortality rates were compared using Student's t-test (SAS, 1992).

Table 2. Composition of the experimental diets used in the third and fourth trial

	Trial 3: Rooster Digestibility			Trial 4: Broiler breeder performance			
Ingredients (%)	Control	Control + Wheat Bran	Control + Arbocel®	Control Male	Arbocel® Male	Control Female	Arbocel® Female
Corn	62.176	61.172	60.566	67.8	68.1	64.15	63.45
Soybean meal	30.091	30.017	30.375	15.0	15.4	25.5	25.7
Arbocel®	—	—	0.800	—	0.8	—	0.8
Wheat bran	—	0.800	—	14.0	12.5	0.8	—
Sunflower oil	3.558	3.838	4.085	—	—	—	—
Soybean oil	—	—	—	—	—	1.0	1.5
Salt	0.443	0.443	0.444	0.3	0.3	0.32	0.32
Limestone	1.71	1.172	1.166	2.0	2.0	7.3	7.3
Dicalcium Pho.	1.856	1.851	1.862	—	—	—	—
Monocalcium Pho	—	—	—	0.6	0.6	0.6	0.6
DL- Methionine	0.250	0.250	0.251	—	—	0.03	0.03
L-Lysine HCL	0.094	0.094	0.088	—	—	—	—
Vit&Min. Mix	0.300	0.300	0.300	0.3	0.3	0.3	0.3
Coccidiostat	0.0625	0.0625	0.0625	—	—	—	—
Calculated							
ME (Kcal/Kg)	3200	3200	3200	2850	2850	2900	2900
CP (%)	20.0	20.0	20.0	15	15	17.5	17.5
TSA (%)	0.88	0.88	0.88	0.50	0.50	0.60	0.60
Lysine (%)	1.10	1.10	1.10	0.88	0.88	0.90	0.90

Results and Discussion

Trial 1: Throughout the trial period, no health issues, pecking or other symptoms were observed. The results on zootechnical performance (weight gains, FC and mortality) did not show any significant difference between the two groups over periods of 1 to 21 days, 22 to 38 days and 1 to 38 days with an average of 2.070 kg live weight at 38 days, 1.667 kg feed/kg live weight gain (FC) and 1.67% mortality (Table 3). In contrast, Sarikhan et al. (2010) found a beneficial effect of lignocellulose on live weight and feed efficiency of broilers at 42 days of age. The apparent discrepancies could be explained by the fact that the latter researchers used "Lohman" strain and lower energy diet (3000 Kcal/kg). Litter moisture level at 21 days show no significant difference with an average of 20.1%. At 38 days, the analyzes showed a significant advantage ($P<0.01$) for the experimental groups (26.6% versus 36.1%). Arbocel® exhibited positive impact at the end of the trial (38 d) indicating that birds are excreting drier fecal material.

Trial 2: The apparent digestibility trial on caged chickens shows drier excreta ($P<0.01$) for the Arbocel® fed group (64.0%) compared to the control group (73.5% humidity). These results are in agreement with Rezaei et al. (2008) who obtained a significant reduction in litter moisture with the use of purified cellulose. Carcass yield increased by +1.1 point and abdominal fat is significantly reduced in the experimental group compared to the control group (1.11% versus 1.23%; $P<0.05$) (Table 3). Improved carcass yield and reduced abdominal fat were also reported by Sarikhan et al. (2010) and could be the result of the current increase in protein digestibility by +5.5 points (62.9% vs 57.4; $P<0.01$). On the other hand, the digestibility of lipids was identical for the two groups (Table 3). The improvement in protein digestibility could be explained by the increase in the length of the intestinal villi (Sarikhan et al., 2010) but also by an increase in the activity of the pancreas which results in an increase in the production of trypsin and chymotrypsin (Bogułsawska-Tryk, 2005). A second hypothesis may suggest the beneficial role of lignin in Arbocel® on the integrity of the digestive system with an increase in favorable bacterial strains such as lactobacilli and bifido-bacteria (Baurhoo et al., 2007).

Table 3. Growth and consumption index (CI), litter moisture, fecal material moisture, protein and fat digestibility % in control and experimental birds

Parameter	Control group	Experimental group	SEM	P value
Weight gain 1-21d (g)	604	591	7.1	NS
FC 1-21d (g/g)	1.380	1.396	0.0213	NS
Mortality 1-21d (%)	0.33	0.50	0.284	NS
Weight gain 21-38d (g)	1427	1442	12.5	NS
FC 21-38d (g/g)	1756	1741	0.0141	NS
Mortality 21-38d (%)	1.34	1.17	0.583	NS
Live body weight 38d (g)	2069	2071	18.1	NS
FC 1-38d (g/g)	1.668	1.665	0.159	NS
Mortality 1-38d (%)	1.67	1.67	0.691	NS
Litter moisture at 21d (%)	20.3	19.9	0.756	NS
Litter moisture at 38d (%)	36.1a	26.6b	1.86	P<0.01
Carcass yield (%)	68.4a	69.5b	0.22	P<0.05
Abdominal fat (%)	1.23a	1.11b	0.035	P<0.05
Fecal material moisture (%)	73.5a	64.0b	0.89	P<0.01
Protein digestibility (%)	57.4a	62.9b	0.40	P<0.01
Fat digestibility (%)	83.0	83.6	1.12	NS

Trial 3. Dietary Arbocel® at a level of 0.8 % had no effect on AME, AMEn, TME, and TMEn (Table 4). However, Arbocel® inclusion caused a significant increase (P<0.05) in true digestibility of protein manifested by a 6% increase in comparison with the control and the control plus 0.8% wheat bran treatments. These confirm our previous findings (Farran et al., 2013) of 5.5% increase in apparent protein digestibility in Ross 308 broilers. Additionally, Arbocel® supplementation resulted in an average increase of 6% (P<0.05) for both apparent and true digestibility coefficients of almost all amino acids. This positive effect of Arbocel® was also observed for the digestibility coefficient of all essential amino acids (Table 5). Previous research work (Boguslawska & Tryk, 2005) demonstrated the association of Arbocel® supplementation in Cobb chicken diets with an increase in proteolytic activity of the pancreatic trypsin and chymotrypsin. Such observations support our findings of improved protein digestion in the current experimental birds. Recently, the work of Yokhana et al. (2016) corroborated results showing increased trypsin activity as well as proteolytic activity of the pancreas. Furthermore, they reported significant increases in both pepsin and intestinal aminopeptidase activities in layer pullets offered control diets supplemented with 1.5% levels of dietary Arbocel®.

Trial 4. The number of eggs, irrespective of their classification (hatching, large, cracked, discarded and table eggs) along with cumulative hen mortality were not significantly different between the two treatments during the six month experimental period. Arbocel® dietary supplementation had the tendency to increase the total number of eggs by 2.68% and hatching eggs by 3.60% which correspond to 3 and 3.8 more eggs per housed hen, respectively. The lack of statistical significance in the effect of lignocellulose on egg production of broiler breeders in the present work and the work of Incharoen and Maneechote (2013), as opposed to those of Lim et al. (2013), may have resulted from differences in the type of birds used in these experiments and/or the period of the feeding trials in each work. Hatchery data on sampled eggs in terms of hatchability and infertility%, collected on a monthly basis throughout the experiment are summarized in Table 6. Infertility% in the Arbocel® treatment group was consistently lower than that of the control in every month the eggs were sampled, but statistical significance (P<0.05) was observed in the 2nd, 3rd, and 4th months of the trial. However, hatchability% of sampled eggs was continuously greater by an average of 4.07% (P<0.05) in Arbocel® fed birds than in control birds. The beneficial impact of Arbocel® on the performance of the breeder hens under investigation could be attributed to the increased protein and amino acid digestibility observed in the current rooster study (Tables 3, 4 and5), and improved nutrient absorption reported by Hetland and Svihus (2001). In addition, the improvement in fertility and hatchability observed in the breeder's trial as a result of feeding lignocellulose may be explained by having a dryer litter (Farran et al., 2013) in Arbocel® houses which could have positively reflected on the health of birds, especially the males that could have less feet problems (Farran et al., 2020), thus allowing them to mate more frequently with females. An average increase in hatchability of 2.81% in the Arbocel® treatment as compared to the control WB treatment was observed during the 6-month trial. Consequently, taking

into consideration the 3.8 increase in hatching eggs along with the 2.81% increase in egg hatchability, one can conclude that 0.8% dietary Arbocel® resulted in 5.6 more saleable chicks per housed hen during a 6 month period. Considering the market price of a control diet (577 $/T), a day-old chick (0.37 $) and market price of Arbocel® average is 1.06 $/kg, then a net profit of 1.812 $ per housed hen is obtained in this commercial operation (Table 7).

Conclusion

The inclusion of Arbocel® in poultry diets significantly improved protein and amino acid digestibility and reduced litter moisture. These results translated into an increase of ready to cook carcass weight of broilers and an enhanced reproductive performance in broiler breeders. In $ value, dietary Arbocel® increased saleable chicks over a six month period with a net profit of 1.812 USD/hen housed.

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Table 4. Averages of apparent and true metabolizable energy (AME and TME) and those corrected for zero nitrogen balance AME_n and TME_n along with protein digestibility coefficients (TCP) in the three diets tested in experiment 1.

Treatment	AME (Kcal/kg)	AME_n(Kcal/kg)	TME (Kcal/kg)	TME_n (Kcal/kg)	TCP (%)
Control	3035	3232	3769	3619	54.3[b]
Control + Wheat Bran	3049	3245	3788	3627	54.0[b]
Control + Arbocel®	3056	3254	3795	3641	60.3[a]
SEM[2]	51.2	41.3	51.2	41.3	1.32

[ab.] Means in the same column with no common superscripts differ significantly (P<0.05).

Table 5. Effect of Arbocel® on apparent dietary amino acid digestibility coefficients[1] (%) of diets and that of essential amino acids

	Apparent dietary amino acid digestibility coefficients (%)				True dietary amino acid digestibility coefficients (%)			
Treatment	Control	Control + Wheat Bran	Control + Arbocel®	SEM[2]	Control	Control + Wheat Bran	Control + Arbocel®	SEM[2]
Aspartate	72.8[b]	72.1[b]	78.5[a]	1.43	85.7[b]	86.0[b]	91.5[a]	1.43
Threonine	58.8[ab]	58.6[b]	65.7[a]	2.60	85.2[ab]	85.0[b]	92.3[a]	2.60
Serine	61.4	60.1	66.4	2.10	86.7	86.6	90.8	2.10
Glutamate	78.3[b]	78.0[b]	82.6[a]	1.14	88.0[b]	88.5[b]	92.5[a]	1.14
Proline	69.6	70.3	73.3	1.61	86.8	88.0	90.8	1.61
Alanine	65.4[b]	66.1[b]	72.6[a]	1.81	83.6[b]	84.9[b]	90.9[a]	1.81
Cystine	48.5	48.5	54.6	2.39	90.5	87.7	90.5	2.39
Valine	63.5[b]	62.6[b]	71.0[a]	2.16	84.9[b]	86.0[ab]	92.4[a]	2.16
Methionine	80.8[b]	81.1[b]	87.6[a]	1.17	89.7[b]	90.4[b]	94.9[a]	1.17
Isoleucine	68.3[b]	67.3[b]	75.0[a]	1.87	85.4[b]	86.0[ab]	92.1[a]	1.87
Leucine	73.4[ab]	73.2[b]	78.5[a]	1.59	86.8[b]	87.2[ab]	92.1[a]	1.59
Tyrosine	67.0[ab]	66.5[b]	73.4[a]	1.87	89.2[b]	89.9[ab]	96.2[a]	1.87
Phenylalanine	73.2[ab]	72.5[b]	78.4[a]	1.61	87.4[b]	87.7[ab]	92.8[a]	1.61
Lysine	76.5[b]	74.3[b]	81.9[a]	1.32	86.0[b]	85.9[b]	91.7[a]	1.32
Histidine	76.3[b]	75.9[b]	81.6[a]	1.60	86.8[b]	86.9[ab]	92.1[a]	1.60
Arginine	77.1[b]	76.5[b]	81.6[a]	1.32	89.5[b]	90.2[ab]	94.5[a]	1.32
Tryptophan	81.1[ab]	79.8[b]	83.9[a]	1.21	90.2[ab]	89.0[b]	93.6[a]	1.21
Essential amino acids	72.9[b]	71.8[b]	78.5[a]	1.60	87.2[b]	87.2[b]	93.0[a]	1.60

[ab,] Means in the same row with no common superscripts differ significantly (P<0.05); [1]There were 9 individual roosters (replicates) per treatment; [2]Pooled standard error of means.

Table 6. Hatchability and infertility % of eggs sampled[1] from broiler breeder hens fed control and Arbocel®) diets over the six months trial

Treatment	Collection period (month)											
	Hatchability						Infertility					
	First	Second	Third	Fourth	Fifth	Sixth	First	Second	Third	Fourth	Fifth	Sixth
Control	73.0[b]	71.8[b]	74.2[b]	66.7[b]	62.9[b]	58.0[b]	7.21	6.52[a]	6.54[a]	4.59[a]	3.53	2.32
Arbocel®	77.1[a]	77.7[a]	76.9[a]	71.1[a]	66.3[a]	62.1[a]	6.32	5.73[b]	4.41[b]	2.88[b]	3.31	2.12
Difference	+4.1	+5.9	+2.7	+4.4	+3.4	+4.1	-0.89	-0.79	-2.13	-1.71	-0.22	-0.20
SEM[2]	1.63	0.9	0.6	1.4	0.66	0.78	0.891	0.232	0.358	0.246	0.114	0.13

[ab] Within a column, averages with no common superscripts are significantly different (P0.05).[1] Each month, a total of 864 eggs were sampled from each house. There were 3 breeder houses/treatment with 4330 hens and 433 roosters/house.[2]Pooled standard error of mean

Table 7. Economics of using Arbocel® in broiler breeder diets taking into consideration the performance results obtained and the current market price in USD ($) of feed ingredients and day-old chicks

Variables	**Control**	**Arbocel®**	**Difference**
Hatching Eggs in 6 months/Hen Housed	105.6	109.4	+3.8 eggs
Hatchability of Total Eggs (%)	68.7	71.5	+2.81%
Number of Chicks Hatched/Hen Housed	72.6	78.2	+5.6 chicks
Feed consumed in 6 months (kg/HH +10% of feed/male)	31.65	31.65	0
Cost of feed ($/kg)	503	511.5	+8.5
Cost of feed consumed in 6 months ($)	15.93	16.19	+0.26
Revenues from the extra 5.6 chicks with a market price of 0.37 $/chick ($)	0	2.072	+2.072
Net profit from chick sale in 6 months ($/Hen Housed)			+1.812

OP21 Organic Minerals in Fast- and Slower-growing Broiler Breeders' Diet on Tibia Characteristics of Their Offspring

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Abstract

This study investigated effects of a combination of inorganic vs. organic macro (calcium and phosphorus) and trace minerals (iron, copper, manganese, zinc and selenium) in the diet of fast and slower-growing broiler breeders on tibia characteristics of their male offspring broilers at similar body weights. Fast- (Ross 308) and slower-growing (Hubbard JA 757) breeders were fed a diet containing inorganic or organic minerals for 10 weeks before egg collection. Eggs were collected and incubated. Male broilers were assigned to 32 pens with 12 chickens per pen. At an average body weight of 1,700 and 2,600 gram, 3 chickens per pen were slaughtered and tibia characteristics were analysed. At a body weight of 2,600 gram in the slower-growing broilers, an organic mineral diet of the broiler breeder resulted in better tibia development with higher thickness (Δ=0.38 cm; P<0.001), osseous volume (Δ=5.1 cm^3; P=0.01) and mineral density (Δ=0.13 g/cm^3; P=0.03) than the inorganic mineral diet, but this was not observed in fast-growing broilers. This suggests that mineral levels in current slower-growing breeder diets might be suboptimal or that transgenerational mineral availability in slower-growing chickens appears to be more effective for bone development, which might be related to longer time that is available for bone development.

Keywords: organic minerals, tibia, broiler breeders, broiler chicken

Introduction

In the last decades, increased attention has been given on improving leg health of broiler chickens, due to an imbalance between high growth rate and immature leg bones, particularly in tibia bone (Knowles et al., 2008; Sherlock et al., 2010; Güz et al., 2019). Suboptimal leg health is known to negatively affect welfare and locomotion, such as accessing water and feed, especially in the last weeks of the broiler rearing period, which can lead to pain, higher mortality, lower slaughter revenues and significant financial losses (Kestin et al., 1999; Mench, 2004; Bessei, 2006; Knowles et al., 2008; Gocsik et al., 2017).

One of the most important factors for bone development is the availability of minerals (Bao et al., 2007; Yenice et al., 2015). Broiler diets mostly consist of an inorganic form of minerals (Van der Klis and Kemme, 2002; Vieira, 2008). However, minerals can also be processed and bound to e.g., an amino acid or a protein and are then called organic minerals (Khatun et al., 2019; Wang et al., 2019). Organic minerals in broiler breeders' diet have been shown to have a higher bio-availability than inorganic minerals, due to a higher chemical stability and a better intestinal absorption (Wedeking et al., 1991; Bao and Choct, 2009;; Bao and Choct, 2009; Torres and Korver, 2018, Wang et al., 2019). Regarding the transgenerational effect, organic trace minerals in the breeders' diet have already been shown to result in better embryonic bone mineralization than inorganic trace minerals (Torres and Korver, 2018).

There were three main reasons for conducting this study: (1) previous research showed that transferring minerals from breeders' to the offspring embryo are only studied for trace minerals, not for macro minerals; (2) effects of mineral source in the breeder diet on offspring bone development at slaughter age are hardly studied; (3) almost all studies on trans-generational effects of minerals in broiler chickens were conducted with fast-growing broiler breeders, not with slower-growing broiler breeders. Therefore, the aim of this study was to investigate effects of inorganic vs. organic minerals in fast and slower-growing broiler breeders' diet on offspring tibia morphological, biophysical and mechanical characteristics.

Materials and Methods

The experiment was setup as a 2 x 2 factorial arrangement with two broiler breeder strains (fast- or slower-growing) and two macro plus trace mineral sources (organic or inorganic) in the broiler breeder diets. The experiment was conducted at the

experimental facility of Wageningen University and Research (Wageningen, The Netherlands). All procedures in this study were approved by the Central Commission on Animal Experiments (The Hague, The Netherlands). 132 fast-growing Ross 308 breeders (120 females and 12 males) and 132 slower-growing Hubbard JA 757 breeders (120 females and 12 males) (both strains were at 20 weeks of age) were used. Breeders were placed in 8 pens (4 pens per strain with 30 females and 3 males per pen). After 5 weeks of adaptation, they were provided with one of two different breeder diets containing either inorganic or organic macro (Ca, P) and trace (Fe, Cu, Mn, Zn, Se) minerals during 10 weeks (from 25 to 34 weeks of age). In the organic diets, Ca and P were largely replaced by Calfos® (Sonac Vuren B.V., Vuren, The Netherlands), a hydroxyapatite form of Ca and P, originating from processed porcine bones. Inorganic trace minerals (Fe, Cu, Mn, Zn, Se) were completely replaced by an organic sourced trace mineral premix (Optimin, Trouw Nutrition, Tilburg, The Netherlands). In the last 5 days of the 34th week of age, first grade eggs were collected and stored. Eggs were then incubated at an eggshell temperature of 37.8^{o}C and relative humidity between 50 to 65% throughout incubation. After hatch, male chickens per broiler breeder strain and diet were randomly assigned to 32 pens in 2 rooms within 8 blocks of 4 pens, and 12 chickens per pen. At a body weight of approximately 1,700 gram (day 29 and 38 of age for fast- and slower-growing chickens, respectively) and approximately 2,600 gram (day 38 and 49 of age for fast- and slower-growing chickens, respectively) 3 chickens per pen were slaughtered and the tibia bones of the right legs were collected. Tibia weight, proximal length, lateral cortex thickness, osseous volume, pore volume, total volume, volume fraction, mineral content and mineral density were analysed on each tibia, using a 3D X-ray microfocus CT scanner (described in Güz et al., 2020, 2021a,b, 2022). The same tibias were then subjected to a three-point bending test. Ultimate strength, yield strength, stiffness and energy to fracture were measured (Güz et al., 2020, 2021, 2022). Tibia characteristics at two body weight classes were subjected to general linear mixed model analysis, using PROC MIXED in SAS (Version 9.4, SAS Institute Inc., Cary, NC, US).

Results

1,700 g Body Weight: In the fast-growing chickens, no effects of mineral source in the breeder diet were found, but slower-growing chickens, originating from organic minerals fed broiler breeders had a higher tibia weight compared to chickens originating from the inorganic minerals fed breeders(Δ=0.86 g on average; P=0.006).

Chickens originating from organic minerals fed broiler breeders had a higher osseous volume (Δ=1.7 cm3; P=0.03), mineral content (Δ=1.1 g; P=0.009), mineral density (Δ=0.07 g/cm^3; P=0.003), ultimate strength (Δ=22.1 N; P<0.001), yield strength (Δ=20.8 N; P<0.001), stiffness (Δ=19.2 N/mm; P<0.001) and energy to fracture (Δ=18.8 N-mm; P<0.001) than chickens originating from inorganic minerals fed broiler breeders.

Slower-growing chickens showed a higher tibia lateral cortex thickness (Δ=0.17 cm; P<0.001), osseous volume (Δ=7.1 cm^3; P<0.001), pore volume (Δ=1.2 cm^3; P<0.001), total volume (Δ=8.4 cm^3; P<0.001), mineral content (Δ=1.9 g; P<0.001), ultimate strength (Δ=37.9 N; P<0.001), yield strength (Δ=39.1 N; P<0.001), stiffness (Δ=39.1 N/mm; P<0.001) and energy to fracture (Δ=35.7 N-mm; P<0.001) than fast-growing chickens.

2,600 g Body Weight: In fast-growing chickens, no effects of mineral source in the breeder diet were found, but slower-growing chickens, originating from organic minerals fed broiler breeders had a higher tibia lateral cortex thickness (Δ=0.38 cm; P<0.001), osseous volume (Δ=5.1 cm^3; P=0.01), total volume (Δ=5.6 cm^3; P=0.005) and mineral density (Δ=0.13 g/cm3; P=0.03) than chickens originating from inorganic minerals fed breeders.

Chickens originating from organic minerals fed broiler breeders had a higher tibia mineral content (Δ=1.4 g; *P*<0.001), ultimate strength (Δ=22.1 N; *P*<0.001), yield strength (Δ=20.8 N; *P*<0.001), stiffness (Δ=19.2 N/mm; *P*<0.001) and energy to fracture (Δ=18.8 N-mm; *P*<0.001) than chickens originating from inorganic minerals fed broiler breeders.

Slower-growing chickens showed a higher tibia weight (Δ=1.65 g; P<0.001), proximal tibia length (Δ=0.30 cm; P=0.02), pore volume (Δ=2.6 cm^3; P<0.001), mineral content (Δ=2.8 g; P<0.001), ultimate strength (Δ=18.1 N; P=0.008), yield strength (Δ=22.6 N; P<0.002), stiffness (Δ=24.6 N/mm; P=0.003) and energy to fracture (Δ=20.0 N-mm; P=0.004) than fast-growing chickens.

Discussion

Results showed that organic macro and trace minerals in breeder diet of slower-growing chickens resulted in better offspring tibia characteristics compared to inorganic minerals in the diet. These findings are in line with previous research, indicating that a higher trace mineral availability in the diet of broiler breeder leads to improved bone development of their offspring (Dibner et al., 2007; Torres and Korver, 2018; Saleh et al., 2019). Replacing inorganic minerals by their organic varieties has been found to positively affect embryonic bone development and later life leg health, probably due to their higher mineral

mobilization and bioavailability (Echigo and Kimata, 2010; Favero et al., 2013; Oviedo-Rondón et al., 2013). In this study, egg and hatchling mineral concentrations of all treatment groups were analysed as well (results were shown in Güz et al., 2022). Because concentrations of minerals in the eggs and hatchlings hardly differed between the organic and inorganic minerals fed broiler breeders, it can be hypothesized that other mechanisms, e.g. mineral availability, have played a role on post-hatch bone development, but these mechanisms are currently unclear. Regarding the strain, slower-growing chickens showed better tibia characteristics compared to fast-growing chickens at both body weights. This difference can probably be explained by the different growth rates and its effects on the speed of bone development. Fast growth is known to result in less mineralised bones, because mechanisms involved in bone development seem to have more difficulities keep up with the fast growth of the broiler (Velleman, 2000; Bonser and Casinos, 2003). Slower growth may ensure that there is more time for bone mineralisation and may compensate for the lack of mineralisation in the early growth phase (Shim et al., 2012; Sanchez-Rodriguez et al., 2019).

Conclusion

Organic macro and trace minerals in the broiler breeder diet showed positive effects on offspring tibia characteristics in slower-growing chickens, whereas this effect was hardly seen in fast-growing chickens, nevertheless the effects on mineral concentration in eggs and hatchlings were limited. This suggests that (1) the difference in feed intake between fast and slower-growing broiler breeders might affect offspring broilers of slower-growing breeders negatively, which might indicate that minerals in current slower-growing broiler breeder diets might be suboptimal or that transgenerational mineral availability in slower-growing chickens appears to be more effective on bone development than in fast-growing chickens, which might be related to the time that is available for bone development. (2) transgenerational mineral availability in offspring appears to play a role via other mechanisms, e.g. mineral availability, than via absolute mineral concentrations in the egg.

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OP22 Probiotic *Bacillus Licheniformis* DSM28710 Supports Broiler-Breeders Under Commercial Conditions

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Abstract

The application of probiotics in broiler-breeders is underrepresented in available research, yet such an application should not be ignored. Probiotic *Bacillus licheniformis* DSM 28710 (B-Act®, Huvepharma) was put to the test under commercial conditions, showing clear benefits: animals that were initially underperforming were able to catch up to the control with the help of the probiotic, whilst they were better equipped to deal with stress situations. Simultaneously, a better functioning of the gastrointestinal tract resulted in a clear reduction of dirty eggs. **Introduction**

Broiler applications dominate the probiotic research in literature. However, broiler-breeders should not be neglected: high-producing hens must also be as efficient as possible, including utilising their diet to the fullest whilst withstanding health challenges. From that perspective the importance of a proper functioning gastrointestinal tract cannot be underestimated, indicating the value a probiotic supplementation can bring to a broiler-breeder operation.

Materials and Method

To evaluate the effect of a probiotic *Bacillus licheniformis* strain in broiler-breeders, a commercial trial was set up. The strain used was DSM 28710 (B-Act®, Huvepharma) and applied via the feed from start to finish.

A total of 27 335 Cobb 500 broiler-breeders were included in the trial: 12 592 female and 973 male birds were assigned to the control group, with the probiotic group consisting out of 12 781 female and 989 male birds. All animals were 29 weeks of age at the start of the experiment, which lasted for 64 days (up until 38 weeks of age). The probiotic group was supplemented with 1.6 x 10^9 CFU *Bacillus licheniformis* DSM 28710 per kg of feed (500 g B-Act®/ton of feed, Huvepharma). Parameters recorded included laying percentage, hatchability and the amount of dirty eggs.

Results and Discussion

When the trial started, animals in the control group had a slightly higher laying percentage compared to the probiotic group (86.31 vs. 84.35%). This difference disappeared halfway the trial, with animals in the probiotic group catching up to the control due to the probiotic supplementation. In regards to hatchability, there was no difference during the trial period itself. That being said, a stress event occurred from week 40 until week 44. Hatchability reduced severely for 4 weeks, but animals previously supplemented with the probiotic *B. licheniformis* were more resilient (less of a reduction in technical performance) and were able to recover quicker compared to animals previously in the control group. In numbers: lowest hatchability for the probiotic group was 79% at week 41, with a partial recovery at week 42 (86.8%) and a full recovery at week 44. The control group had a low point hatchability of 74.4% at week 42, with a full recovery at week 44 as well.

In terms of dirty eggs for the trial period, animals supplemented with probiotic *B. licheniformis* DSM 28710 clearly benefitted from the addition to their diet: only 943 eggs were recorded as dirty eggs, compared to the control's 1780 – a reduction of almost 50%. This is in line with earlier research, conducted both in layers as well as broilers: both applications noted an improved functioning of the gastrointestinal tract, indicated by a reduction in dirty eggs (layers) and reduced wet litter (broilers). A secondary layer trial with the same probiotic also looked into manure parameters, including moisture and protein levels. A significant decrease in excreta moisture was noted in the probiotic group (moisture reduction of 5.3% compared to the control's 4.5%; $P<0.05$), which further lends credibility to the hypothesis that *B. licheniformis* DSM 28710 supports a better functioning of the gastrointestinal tract in layers and broiler-breeders. This includes an improved usage of the provided nutrients from the diet, as highlighted by the final manure parameter: a significant reduction in manure protein in the probiotic group was noted (20.5 vs. 24.1% in the control, $P<0.05$), indicating that nutrients were utilised better when the probiotic was present.

Conclusion

The results here clearly show that *B. licheniformis* DSM 28710 supports high-performing broiler-breeders under stressful commercial conditions. As a result, animals were better equipped to deal with these conditions, whilst a better functioning of the gastrointestinal tract resulted in a clear reduction of dirty eggs. The results obtained under commercial conditions were in line with previous research on *B. licheniformis* DSM 28710 in layers and broilers, where similar improvements in secondary feed-efficiency parameters were noted. Probiotic *B. licheniformis* DSM 28710 should thus be included in diets formulated for high-performing broiler-breeders, to ensure optimal feed efficiency and animal resilience to stressful conditions.

IS09 Structure and Function of the Enteric Immune System and Its Role in Enteric Diseases

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Abstract

Development of the immune system is controlled by genetic programs and environmental cues. While mammals acquire the initial gut flora from their mother this is not the case in modern poultry production. Whether this impacts on the development and functional maturation of the immune system is largely unclear. We therefore compare immune system development in sterile (germ free, GF), mono- and tetra-reconstituted and SPF birds as well as birds raised in the presence of a maternal flora.

No significant differences were evident in the development of selected parameters of the innate immune system between the groups. In contrast, by using microarray based gene expression studies striking differences were observed in the adaptive immune system. In particular, mRNA abundance of genes associated with the B-cell system was significantly reduced in GF birds. This was reflected by highly reduced numbers of B-lymphocytes in the gut, which was paralleled by a complete absence of IgA production. The B-cell compartment and IgA production were slightly restored by mono-reconstitution and further enhanced in tetra-reconstituted and SPF birds. However, development of the B-cell system was greatly retarded in all groups in comparison with birds that acquired a maternal flora on the day of hatch. In contrast, neither B-cell maturation in the bursa of Fabricius nor circulating B-cell numbers were affected. These data predict that molecular cues induced by microbial colonization attract circulating B-cells into the mucosal tissue and regulate maturation towards IgA producing cells. Interestingly, these signals do not only activate homing and maturation of the B-cell compartment but also maturation of the epithelial IgA transporter system as poly-Ig receptor (PIGR) expression was very low in germ free birds but induced in response to microbial colonization. Identification of these microbiota derive signals will be an important future task.

Introduction

The mucosal surface of the gut is a fascinating structure. A single epithelial barrier separates the intestinal content from the body. The intestinal content does not only consist of digested feed but harbors a highly complex microflora called the microbiome. Consisting of bacteria, fungi and archaea and some poorly defined viruses the microbiome has been estimated to be made up of more than 1000 different species collectively representing more than 100-fold more cells than the vertebrate host itself does. The microbiota is well known for its capability to digest plant products which otherwise would be inaccessible for the vertebrate as a nutrient source. However, research in recent years has demonstrated that the microbiome affects not only metabolic processes but also the development and function of the immune system (Belkaid and Hand 2014). Conversely, the composition of the microbiome seems to be modulated by the immune system. This cross-talk between the bacterial flora, the intestinal epithelium and the immune system has become a research focus in immunology over the last 10 years. Immunologists have used germ free mouse models, gnotobiotic mice and mice treated with antibiotics to get completely sterile animals or mice with a defined microbiome. This work has led to a wealth of information in mouse models (Kamada and others 2013) while knowledge in humans and domestic animal species is still limited.

The Avian Immune System

The avian immune system is largely similar to its mammalian counterpart, even though some striking differences are found such as the absence of lymph nodes and the presence of a unique organ for B-lymphocyte development only existing in birds named the bursa of Fabricius (Schat and others 2014). Two major arms of the immune system have been defined which are called the innate and the adaptive immune system. The innate immune system provides a rapid response to pathogenic challenge while the adaptive immune system is responsible for sustained pathogen control and immunological memory. Both systems are made up of cellular and soluble components some of which have been known for a long time including macrophages and heterophils, complement components, and antibodies. Others have been characterized more recently in mice but are still poorly defined in birds (e.g. T-lymphocyte subsets or innate lymphoid cells). The two arms of the

immune system closely interact with each other to provide adequate protection and communicate with each other through cell surface molecules and soluble factors collectively called cytokines. Cytokines play a critical role in the regulation of immune response to pathogens, vaccination, in immuno-regulation, and in the control of inflammation. Since the initial publication of the chicken genome, significant progress has been made in avian immunology. Genomic data provides sequence information for genes involved in immuno-regulation and the function of the well-defined lymphoid tissues of the spleen, thymus, and the bursa of Fabricius. In contrast, mucosal tissues of the lung, reproductive tract, and the gut are less well understood.

Development of the Gut Associated Innate Immune System of Chickens

Using immunohistology, holistic gene expression analysis, and functional assays we have investigated the development of the gut immune system from hatch to seven weeks of age in both broilers and layers. Macrophages are readily found in large numbers from the day of hatch throughout the gut. Their number increases in parallel with gut growth (Figure 1). Macrophages are located in the lamina propria and are thus well positioned to rapidly detect invading pathogens or epithelial damage. Studies in *Salmonella enterica* infection models of day old chicks have revealed that these macrophages are fully functional and respond to pathogen invasion with the secretion of inflammatory cytokines and chemokines to attract more phagocytes into the mucosal tissue which help to clear the infection. Within hours of infection the chemokines interleukin-8 (IL-8) and CCL-16 are produced. They attract heterophils and monocytes from the blood, respectively. In addition, effector cytokines regulating the anti-microbial response such as interferon-γ have been detected by RT-PCR. Thus, macrophages and other components of the innate immune system are present and functional at hatch when the bird first encounters microbial organisms in its environment.

In addition to the cellular components of the innate immune system, soluble components are found early after hatch. These include several acute-phase molecules of the pentraxin family and anti-microbial peptides of the defensin family (Cuperus and others 2013), which have been demonstrated by RT-PCR analysis. However, their functional role in the avian intestine has not been studied due to the lack of appropriate animal models deficient on one or several of these factors. From work in mice it has become clear that anti-microbial peptides are produced by epithelial cells of the crypts called paneth-cells and are secreted into the mucus layer. Bacteria invading the mucus barrier get in contact with these factors and are efficiently lysed. Most authors agree that paneth-cells (Nile and others 2004) are absent in chickens and even though defensin specific mRNA is found in gut tissue samples it is largely unclear where they are produced and which mechanisms elicit their synthesis and secretion. However, recent work described new gut epithelial cell culture models and identified paneth cells by their capacity to produce lysozyme (Orr and others, 2021).

Development of the Gut Associated Adaptive Immune System of Chickens

While the innate immune system seems to be functional at hatch the components of the adaptive immune system are absent at that time point. Developmental studies have shown that B-lymphocytes, the producers of antibodies, start to emigrate from the bursa of Fabricius around hatch. Similarly, emigration of T-lymphocytes from the thymus starts shortly before hatch but major waves of cell migration are only observed days after hatch (Ratcliffe 2006). Importantly, both populations of lymphocytes are rare in the gut tissue during the first two weeks after hatch (Figure 2). Subsequently, more and more lymphocytes colonize the mucosal tissues and formation of lymphoid aggregates is found. Lymphocytes can be demonstrated in the lamina propria and in the epithelium as intra-epithelial lymphocytes. The latter population is made up of T-cells specialized in the control of virally infected cells. They are called cytotoxic T-lymphocytes (CTL) and are characterized by the expression of the cell surface protein CD8. T-helper cells (expressing the CD4 molecule) are more restricted to the lamina propria and are found in close proximity to lymphoid aggregates. B-lymphocytes start to colonize the gut at nearly the same time. They form organized lymphoid structures called Payer's patches and caecal tonsils.

Studies performed more than 50 years ago with birds raised under germ free conditions have shown that bacterial colonization has a significant influence on the maturation of the gut associated immune system (Thorbecke and others,1957). This observation has been confirmed recently in germ free mouse models and in our own studies with germ free chickens. To investigate the role of the microbiota, we hatched groups of birds which were maintained sterile or received one (*E.coli* Nissle) or a mixture of four strains of bacteria (*E.coli, Lactobacillus, Enterococcus, Clostridium*). These birds were compared with chickens housed under SPF conditions. Even in the complete absence of microbiota the innate immune system (as far as investigated) developed normally. No difference was observed with regards to colonization by macrophages and micro-array studies showed undisturbed expression patterns for several genes of the innate immune system. In striking contrast, the development of the adaptive immune system was strongly impaired. B-cells were absent in the lamina propria of four weeks old birds despite normal B-cell development in the bursa of Fabricius. B-cell regions of the caecal tonsils were hugely

underdeveloped and did not contain germinal centers (Figure 3), which are essential for the production of IgA and IgY antibodies. Consequently, IgA production was absent in germ free birds in the gut and in the serum (Figure 4). A similar picture was observed in the T-cell system with the absence of both CD4+ and CD8+ T-cells in germ free animals. Colonization with *E.coli* alone did not revert the histological picture but lead to the production of IgA in serum. These antibodies were, to a large extend, *E.coli* specific. Birds colonized with four bacterial strains showed significant maturation of the adaptive immune system in the gut. This was most pronounced in the caecal tonsils were germinal centers formation and IgA production was observed. The described observations were further supported by our gene expression analysis. Comparison of micro-array data from germ free and SPF birds showed higher expression of a large number of genes associated with immune function and developmental processes in SPF chickens. Most strikingly, genes known to be critical in B-cell maturation were found to be far less abundant in germ free birds reflecting the phenotype observed by immune-histology. AID, an enzyme essential for immunoglobulin class switching from IgM to IgA and IgY was weakly expressed in germ free birds but significantly higher expressed in mono-reconstituted chickens. A further increase was detectable in tetra-reconstituted animals, which showed expression levels equivalent to SPF birds.

Response toInfection

Three defense layers can be distinguished at the mucosal barrier.

1. Mucus secreted by goblet cells prevents many pathogens and commensal bacteria to gain access to the gut epithelial cell layer. The mucus membrane not only provides a physical barrier but also binds antimicrobial peptides and IgA to enforce its function. However, it should be noted that some pathogens use the mucus as a source of nutrients.
2. The second layer is made up of the gut epithelial cells, which provide a physical barrier and secret antimicrobial peptides. Importantly, epithelial cells communicate with their environment through receptors sensing microbial molecules, so called microbe associated pattern recognition receptors (MAMP-R). Upon activation of such receptors, epithelial cells start to secret a range of signaling molecules, which modulate the function of the immune system. One example is the recruitment of heterophils upon epithelial IL-8 secretion, a hallmark of mucosal inflammation. Other signaling molecules inform the adaptive immune system, thus initiating targeted responses of T-cells to the respective pathogen.
3. Once pathogen have succeeded to overcome the mucus and epithelial barriers, they encounter the enteric immune system. As discussed before, the gut immune system comprises both, cells of the innate and of the adaptive immune system. Cells of the innate immune system are first responders, which recognize the presence of pathogens and initiate an adequate response. *Lamina propria* macrophages are particularly important, since they express numerous MAMP-Rs and can secret a vast variety of cytokines and chemokines. However, under homeostatic conditions, this system exhibits anti-inflammatory properties. It thus prevents an inadequate response to intrusion of commensal bacteria or irrelevant numbers of pathogens, both of which will be cleared by phagocytosis and intracellular destruction. On the other hand, if pathogens find their way into the *L. propria* posing a threat to the host, macrophages will respond by sending alarm signals to recruit immune cells to the tissue and to mount an inflammatory response. Depending on the pathogen, this response will activate different arms of the adaptive immune system. So called T_{helper}-1 (Th1)(cells secret the cytokine interferon-γ to control intra-cellular infections (Salmonella, Yersinia, Mycobacteria, Coccidia). Extracellular bacterial infections, such as APEC, will be controlled by the activation of Th2 cells helping B-cells to make antigen specific antibodies. Clearance of such pathogens is further achieved through Th17 cells, which produce IL-17 to recruit heterophils from the blood stream.

Trace mineral Premix

Calcium (Ca) Min.	3.20%
Calicium (Ca) Max.	4.20%
Iron (Fe) Min.	2.63%
Magnesium (Mg) Min.	2.68%
Maganese (Mn) Min.	13.40%
Zinc (Zn) Min	10.70%
Copper (Cu) Min	4000 ppm
Iodine (I) Min.	1000 ppm
Selenium (Se) Min.	400 ppm

Vitamin Premix

Vitamin A, I..U./LB	1,000,000
Vitamin D3, I..U./LB	200,000
Vitamin E, I..U./LB	2,000
Vitamin B-12, MG./LB	2.20
Riboflavin, MG./LB	800
Niacin, MG./LB	8,000
d-Pantothenic Acid, MG./LB	2,000
Choline, MG./LB	34,720
Menadione, MG./LB	132
Folic Acid, MG./LB	100
Thiamine, MG./LB	400
Pyridoxine, MG./LB	400
Biotin, MG./LB	20

From this brief overview, it becomes clear that T_{helper} cells are critical in initiating and modulating an appropriate immune response. A lack or inhibition of these cells by e.g. pathogens, mycotoxins or stress responses leads to immuno-suppression and disease. On the other hand, an overwhelming and lasting T-cell response may cause chronic inflammation or auto-immunity, both of which have to be prevented. Regulatory T-cells are essential in achieving this balance between the control of infection and prevention of immune-pathology. While Th1, Th2 and Th17 cells have been characterized in chickens in more detail, T_{reg} cells are still poorly understood, despite their importance as demonstrated in murine models.

Conclusion

The development of the gut immune system is controlled by both genetic and environmental factors. The innate immune system develops early in ontogeny and is present at hatch and thereby able to protect birds during the critical phase of sudden encounter with microbes after hatch. Its subsequent development is not strongly influenced by the microbiota but it becomes strongly activated in response to pathogen invasion. In contrast, development of the adaptive immune system is significantly influenced by bacterial colonization of the gut. Absence of a microbiom leads to severe developmental defects with a lack of B- and T-lymphocytes in the gut mucosa and the absence of antibody formation.

Our studies strongly indicate that full maturation of the gut immune system requires a complex flora. However, the precise composition of an optimal flora is still unknown. Under natural conditions hatchlings get in immediate contact with the maternal flora while birds hatched under commercial poultry production conditions are hatched essentially sterile. It is still unclear how these conditions influence the development of the mucosal immune system, the capability to control pathogenic microorganisms, and animal health and welfare. Interestingly, mouse studies have shown that nutrients and fermentation products also influence the functional status of the mucosal immune system either directly or through mediators secreted by the epithelial cells. The role of the epithelium, the mucus barrier, and macro- and micro-nutrients on the avian immune system has not been investigated to date but should be considered in future studies.

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OP23 Phytogenics: An Effective Alternative Solution For Better Gut Health in Broilers

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Abstract

In recent times, trends for antibiotic-free poultry production are driven by legislative guidelines and consumer pressure. To maintain broiler performances while reducing dependency on antibiotics focus on gut health is becoming critical. The gut health of broilers has broad implications on the overall health of birds and the production efficiency of flocks. Phytogenic feed additives could be an effective alternative solution to maintain performance in broilers with reduced use of antibiotics

Keywords: ABF, Antibiotics, Phytogenics, broilers

Introduction

Commercial broiler operations have undergone enormous changes in production practices over the last 5 decades. Genetic selection for high production rates along with improved management techniques and dietary measures have led to increased performance standards in all poultry operations. However, the scientific community is unanimous on focusing on gut health if we want to achieve high performance consistently.

A healthy avian gut is essential to optimize digestibility, minimize nutrient excretion, and consequently mitigate the environmental impacts of ammonia, odours, and other gas emissions with health and welfare impacts inside and outside the poultry house that can impact the health and welfare of birds and human workers (Nahm, 2002; Costa et al., 2008). The production of antibiotic-free (ABF) poultry is an increasing trend worldwide (Cervantes, 2015) because the use of some antibiotic growth promoters (AGPs) has been banned by certain governments in many countries (Phillips, 2007) and consumer interest in avoiding the consumption of food products that may contain traces of AGP is increasing in others (Phillips et al., 2004; Brewer and Rojas, 2008). The current study aimed to evaluate the effects of Phytogenic Feed Additive (PFA) on the performance of broilers in high gut-challenged conditions.

Materials and Methods

The study was conducted at Southern Poultry Feed & Research (SPFR), Athens, GA (USA) over a period of 42 days.

The experimental house was divided into pens of equal size, arranged along a central aisle. The birds were kept in 22 pens of the house each having an area of 4 x 10 = 40 ft^2, with built-up litter as bedding with a thickness of approximately 4 inches. The stocking density, after subtracting out for equipment, was 0.83 ft^2/bird. Each pen has 5 feet high side walls with the bottom 1 1/2 feet being of solid wood to prevent bird migration.

The temperature & relative humidity of the building was monitored. Environmental conditions during the trial (temperature & humidity) were appropriate (optimum) for the age of the birds. Illumination was provided by fluorescent bulbs placed above the pens. The lighting scheme was 22 light and 2 dark hours per day. The diets were provided *ad libitum* in one tube-type feeder per pen. From day 1 until day 7, the feed was also supplied on a feeder tray, and placed on the litter of each pen. Water was provided *ad libitum.*

Standard floor pen management practices were used throughout the experiment. Animals and housing facilities were inspected twice daily, observing and recording the general health status, constant feed, and water supply as well as temperature, removing all dead birds, and recognizing unexpected events. Birds found dead during the study were noted on the Daily Mortality Record and were not replaced. Pen number, the date of mortality, sex, weight, and diagnosis were recorded.

Birds : 880 Day old Cobb 500 male chicks were allocated to the study. Birds were divided into two dietary treatments with 11 replicates and 40 birds each replicate; Control (standard diet formulation) and PFA (control + PFA @ 100gm/MT of feed).

At the hatchery, the birds received routine vaccinations. Only healthy-appearing chicks were used in the study. At the start of

the study, forty chicks were allocated to each treatment pen by blocks. No birds were replaced during the course of the study. Bird weights (kg) by pen were recorded at Day 0, Day 21, and Day 42. Birds found dead during the study were noted on the Daily Mortality Record, and were not replaced. Pen number, the date of mortality, sex, weight, and diagnosis were recorded

Diets :All feeds were fed as crumbles/pellets. Quantities of all basal feed and test articles used to prepare treatment batches were documented. Each batch of feed was mixed and bagged separately. Each bag was identified with the study number, date of the mix, type of feed, and the correct treatment number. Complete records of feed mixing and test article inventories were maintained.

All feed was weighed by pen. Starter feed was fed from Day 0 to 21. On Day 21, the non-consumed starter was weighed and discarded. Grower feed was issued and fed until Day 42. On Day 42, the non-consumed Grower was weighed and discarded

Table 1. Diet formulation (%, as fed)

Ingredient	**Starter %**	**Grower %**
Corn, yellow, grain	52.31	54.55
Soybean meal dehulled, solvent	36.83	30.57
De oiled DDGS	5.00	8.00
Fat, vegetable	2.51	3.82
Dicalcium phosphate.	1.12	1.00
Calcium carbonate	1.01	0.96
Methionine MHA	0.37	0.31
Salt, plain (NaCl)	0.36	0.35
L - LYSINE	0.25	0.23
L-Threonine 98.5	0.09	0.06
Trace Mineral	0.08	0.08
Vitamin premix	0.07	0.05
Quantum blu	0.01	0.01
Calculate Analysis	**Starter**	**Grower**
[volume]	100.00	100.00
Protein, crude	22	20
Fat, crude	4.92	6.44
Fiber, crude	2.44	2.51
Calcium	0.9	0.84
Phos. Total	0.62	0.58
Phos., available	0.45	0.42
M.E. poultry	3,008.00	3,086.00
Sodium	0.18	0.18
dig methionine	0.61	0.54
dig cysteine	0.25	0.24
dig lysine	1.18	1.05
dig tryptophan	0.27	0.24
dig threonine	0.78	0.69
dig isoleucine	0.86	0.77
dig TSAA	0.88	0.80

Table 2. Premix

Sampling and gut lesion scoring : On Day 21 and Day 35, three birds per pen were sacrificed and GIT was scored for necrotic enteritis. Scoring system for experimental necrotic enteritis lesions proposed for international adoption (Shojadoost *et al*, 2012)

Score	Lesion	Number of lesions
0	No gross lesions	-
1	Thin or friable walls, or diffuse superficial but removable fibrin	-
2	Focal necrosis or ulceration, or non-removable fibrin deposit	1 to 5 foci
3	Focal necrosis or ulceration, or non-removable fibrin deposit	6 to 15 foci
4	Focal necrosis or ulceration, or non-removable fibrin deposit	16 or more foci
5	Patches of necrosis 2 to 3 cm long	Variable
6	Diffuse necrosis typical of field cases	Variable, but extensive

Data entry and analysis **:** Source data were entered with indelible ink. Entries were legible, signed or initialed, and dated by the person making the observation entry. Each sheet of source data was signed by the person(s) attributed to the data. Any mistake or change to the source data was initialed and dated and a correction code or statement was added as to why the change was made.

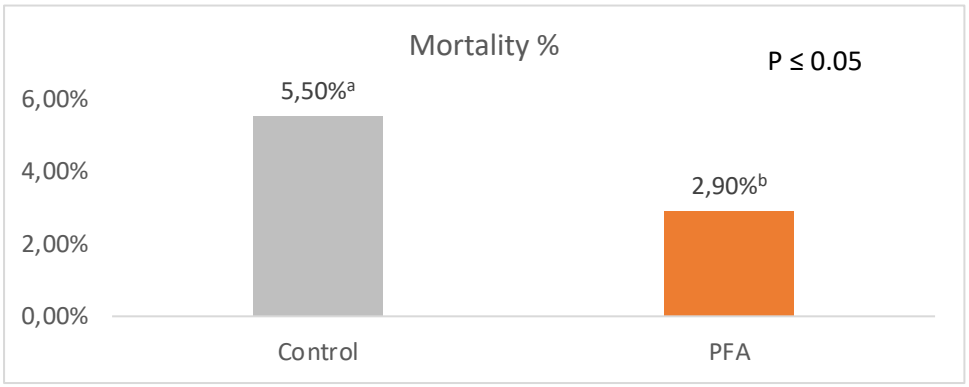
The raw data were analysed statistically (ANOVA) using a Random Complete Block Design. Tukey's HSD test ($p \leq 0.05$) was used to separate means when ANOVA F values are significant ($p \leq 0.05$).

Disposal of birds and feed : All birds and feed were buried in SPFR's pit as described in SPFR SOPs. Records of disposition were included in the source data.

Results and Discussion

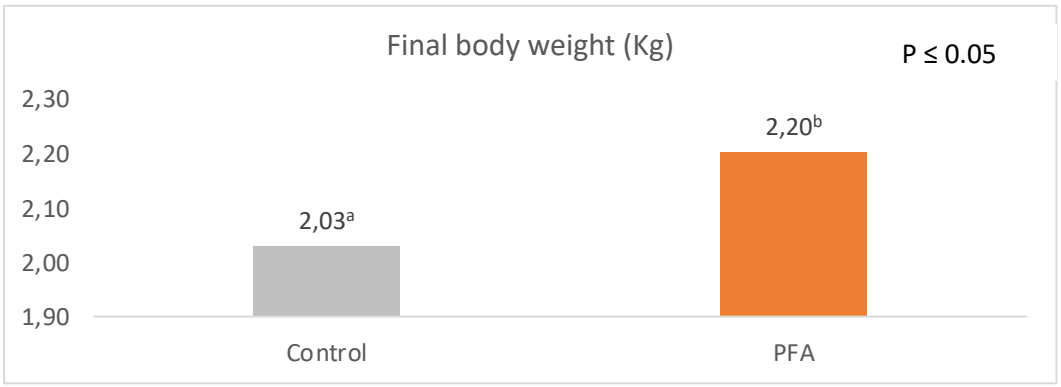
The results were analyzed for key performance parameters like mortality %, average body weight, feed conversion ratio, and also for gut lesion scoring for necrotic enteritis.

Figure 1: Mortality (%) observed in control and PFA groups at D42



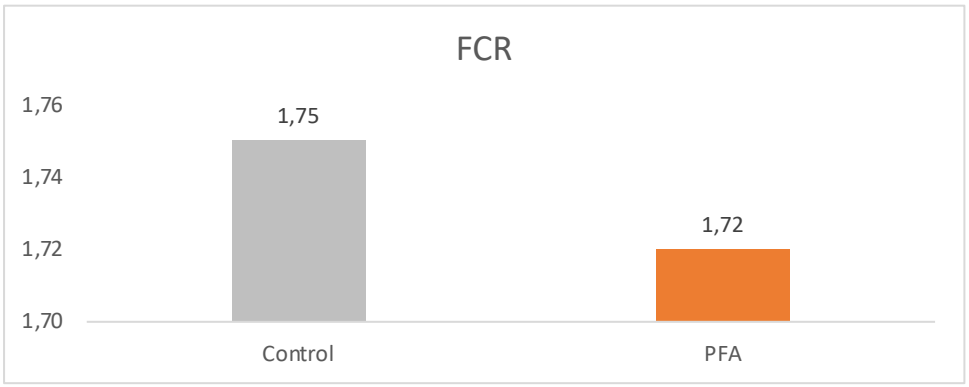
As shown in figure 1, at 42 days of age the PFA group supplemented group, showed 2.6% less mortality than the control group which was significantly different at P value ≤ 0.05.

Figure 2: Average body weight (Kg) recorded in control and PFA group at D42



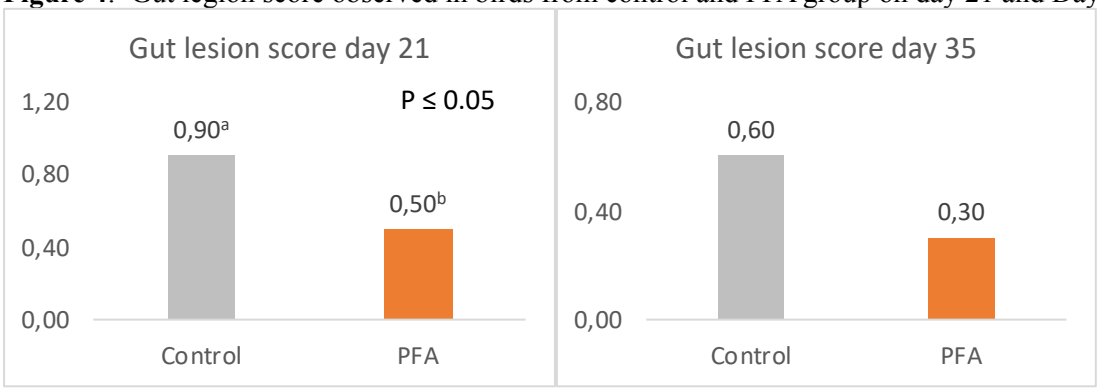
The control group birds attained 2.03 Kg average weight in comparison to 2.20 Kg in the PFA group at 42 days (figure 2) with a significant difference at P value ≤ 0.05. The average daily gain was 47 gm in the control vis-à-vis 51 gm in PFA.

Figure 3: Feed conversion ratio over the complete study period (0-42 days) in both control and PFA groups



The feed conversion ratio of the PFA group was 3 points better than control groups (figure 3)

Figure 4: Gut legion score observed in birds from control and PFA group on day 21 and Day 35



The gut lesion score is an effective tool to assess subclinical necrotic enteritis. As shown in figure 4, for the PFA group the NE gut lesion score was significantly 44% lower on day 21 (P value ≤ 0.05) and 50% lower on day 35 in comparison to the control.

Discussion and Conclusion

Phytogenic additives are known for their antimicrobial, anti-inflammatory, and antioxidant activities which support better gut health during highly challenging conditions. In the current study, the PFA showed significant improvement in performance parameters like mortality and average body weight in broilers. PFA also helped reduce the NE gut lesion score which indicates, that PFA could able to lower inflammation in the gut and contribute to a better feed conversion ratio.

As the trend towards antibiotic-free poultry production gains momentum, a concerted focus on supporting birds' gut health is key to achieving optimal performance. In combination with good dietary, hygiene, and management practices, phytogenic additives can be considered a potent tool for reducing the use of antibiotics.

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OP24 Evaluation of the Effect of MCFA on the Expression of Avian Beta-Defensin Genes During A *Salmonella* Infection in Broiler Chickens

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Abstract

Medium chain fatty acids (MCFA) are known for their strong direct antimicrobial activity. MCFA may, however, also indirectly contribute to better animal health and disease resistance by modulating host immune cells.

In this experiment we investigated if a specific MCFA blend could influence the expression of avian β-defensin genes during a *Salmonella* infection in broiler chickens. A total of 144 male Ross 308 birds were used for this study. Animals received drinking water and feed *ad libitum* throughout the entire study. *Salmonella*-negative chickens (sentinels) were randomly distributed per 20 birds to 6 isolator units. Half of the sentinel birds received a standard control diet, the other half with 600 gram/ton MCFA added on top. Seeder birds were housed separately and orally inoculated with 10^9 CFU of a virulent *Salmonella* Enteritidis strain (SE U.09.76) at 3 days of age and received a standard control diet. At 8 days of age, 4 *Salmonella*-positive birds (seeders) were put into each isolator to assure a gradual *Salmonella* transmission to the sentinels. At 20 days of age, all birds were euthanized and from the sentinels small pieces (0.5 x 0.5 cm) were collected from the oesophagus, duodenum (tip of the loop) and spleen. Samples of each isolator were randomly pooled (n = 5 samples/isolator) and grouped per treatment (n = 3x5 samples/treatment). The relative gene expression levels of *AvBD1*, *AvBD7* and *AvBD9* were analyzed with qPCR by normalizing the expression against housekeeping genes *RPL10* and *TBP*. Data were analyzed by ANOVA, using the general linear model procedure of R. The statistical model included the fixed effect of treatment. Differences were considered significant at $p < 0.05$, and in that case were followed by a Tukey multiple comparison test.

A significantly increased *AvBD9* expression was observed in the spleen of sentinel birds receiving MCFA compared to the negative control group ($p = 0.038$), which shows the potential of these molecules to infer also systemic effects in poultry. An MCFA-induced expression of *AvBD9* in chicken immune cells has already been shown *in vitro*.

These data show that dietary MCFA can influence the expression pattern of avian β-defensins during a *Salmonella* colonization in broiler chickens, through which they could be a valuable tool in controlling *Salmonella* in live poultry resulting in less contaminated broiler meat.

Introduction

Today, salmonellosis is the second most reported zoonosis in humans (after campylobacteriosis) in the European Union and poultry products such as meat and eggs pose a great threat [1]. To efficiently reduce salmonellosis in humans, the potential transmission of pathogens from poultry should be minimized as much as possible. Several intervention strategies exist and are implemented today already, but natural products that control *Salmonella* colonization in chickens during primary production in a cost-effective way are scarce.

MCFA are known to exert strong antibacterial effects against a broad range of bacterial pathogens and viruses, including *Salmonella* [2]. Next to a direct antibacterial effect by disrupting the bacterial cell membrane [3], MCFA can also reduce the virulence of *Salmonella* by reducing its invasion potential in gut epithelial cells and as such lowering gut colonization and translacotion to organs (such as liver and spleen) of this bacterium [2].

MCFA have also been shown to upregulate the expression of avian beta-defensins in chicken immune cells [4]. Beta-defensins are small peptides that belong to the innate immune system and have a wide antibacterial action [5]. As such, MCFA may also indirectly contribute to reduced *Salmonella* colonization in poultry.

Materials and Method

In this experiment we investigated if a specific MCFA blend could influence the expression of avian β-defensin genes during a *Salmonella* infection in broiler chickens. A total of 144 male Ross 308 birds were used for this study. Animals received drinking water and feed *ad libitum* throughout the entire study. Chickens negative for *Salmonella* as proven by cloacal swabbing (sentinels) were randomly distributed per 20 birds to 6 isolator units. Half of the sentinel birds received a standard control diet, the other half were fed the same diet with 600 gram/ton MCFA added on top. Seeder birds were housed separately and orally inoculated with 10^9 CFU of a virulent *Salmonella* Enteritidis strain (SE U.09.76) at 3 days of age and received a standard control diet. At 8 days of age, 4 *Salmonella*-positive - as proven by cloacal swabbing - birds (seeders) were put into each isolator to assure a gradual *Salmonella* transmission to the sentinel birds. At 20 days of age, all birds were euthanized and from the sentinels small tissue pieces (of 0.5 x 0.5 cm) were collected from the oesophagus, duodenum (tip of the loop) and spleen. Samples of each isolator were randomly pooled (n = 5 samples/isolator) and grouped per treatment (n = 3x5 samples/treatment). The relative gene expression levels of *AvBD1*, *AvBD7* and *AvBD9* were analyzed with qPCR by normalizing the expression against housekeeping genes *RPL10* and *TBP*. Data were analyzed by ANOVA, using the general linear model procedure of R. The statistical model included the fixed effect of treatment. Differences were considered significant at $p < 0.05$, and in that case were followed by a Tukey multiple comparison test.

Results and Discussion

In general, beta-defensins were more highly expressed in the spleen compared to the oesophagus and duodenum. Expression of *AvBD9* was - irrespective of treatment group - significantly lower in the duodenum and spleen compared with *AvBD1* and *AvBD7* expression, while the opposite was true in the oesophagus (data not shown). Although slight numeric differences in the expression of avian beta-defenins could be noted in the eosophagus and duodenum between sentinels animals fed a control diet or a diet with MCFA on top (data not shown), none of these were significant. In the spleen, however, a significant ($p = 0.038$) 4-fold increase in *AvBD9* expression was observed in sentinel birds receiving MCFA compared to those fed the control diet (see **Figure 1**). The observation that dietary MCFA can modulate the beta-defensin expression profile in the spleen of broiler chickens shows the potential of these molecules to infer also systemic effects in poultry.

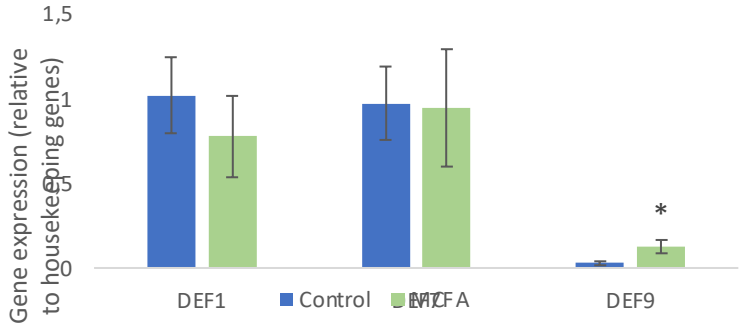

Figure 1 Avian beta-defensin 9 (AvBD9) expression in the spleen of 20-d old broiler chickens

The MCFA-induced expression of *AvBD9* observed in this study is a confirmation of previous *in vitro* research performed in chicken immune cells, where also an increased expression of *AvBD9* was observed in HD11 macrophages and primary monocytes after 24h incubation with MCFA [4]. However, we observed neither a reduction in *Salmonella*-positive sentinels nor in the mean probable number (MPN) in the sampled organs. Potentially, the model used in this study was too aggressive in order to see a beneficial effect of the MCFA on controlling *Salmonella*.

Nevertheless, these data show that dietary MCFA can influence the expression pattern of avian β-defensins during a *Salmonella* colonization in broiler chickens, through which they could be a valuable tool in controlling *Salmonella* in live poultry, potentially leading to less contamination of broiler carcasses during slaughter.

Conclusion

This study shows that dietary MCFA can influence the expression pattern of avian β-defensins during a *Salmonella* colonization in broiler chickens. Further research is needed to examione to what extent an MCFA-induced splenic expression of *AvBD9* might assist in *Salmonella* control in poultry.

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OP[25] Trials on the Addition of *Saccharomyces cerevisiae* Boulardii and its' Effects in Broilers

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Abstract

Saccharomyces cerevisiae boulardii (SCB) is a probiotic yeast used as a dietary supplement in broilers. The aim of our study was to determine the effects of different doses of SCB added in feed or water on production performance, hematology and biochemistry parameters, intestinal microflora, and intestinal microbiota in broilers. For this purpose we performed two stages of field and laboratory investigations. The first stage trial was done on four groups. Group 1 didn't receive SCB in the feed, group 2 received 150 g/1000 kg of SCB $1,5x10^{10}$ CFU/g, group 3 received 500 g/1000 kg of SCB $1,5x10^{10}$ CFU/g (0,05%) and group 4 received 1000 g/1000 kg of SCB $1,5x10^{10}$ CFU/g. The second stage trial was done on three groups. Group 1 received 500 g/1000 kg of SCB $1,5x10^{10}$ CFU/g in feed, group 2 didn't receive SCB either in the feed or water (control group), and group 3 received 250 g/1000 l of SCB $1,5x10^{10}$ CFU/g in drinking water. The FCR and EPEF values indicate a statistically significant difference ($p<0,05$) between the control group and the SCB treated groups. G3 had the best values for these two parameters. Albumins, total proteins, globulins, cholesterol, triglycerides, urea and calcium were significantly lower ($p<0,05$) in G4 in comparison with G1 and G2. While, ALT and AST were significantly higher ($p<0,05$) in G3 and G4. In the second trial, the G1 had the lowest FCR (1.49) compared to G2 (1.64) and G3 (1.61) and the highest EPEF (448) compared to G2 (386) and G3 (423). The G3 had the highest final body weight (2861 g) compared to other groups. Considering the microbiome profiling data, the predominant fecal populations were Bacillaceae and Thermoactinomyces for G1, Bacillaceae, Thermobacillus and Brevibacillus for G2 and Bacillaceae, Thermoactinomyces and Rickenellaceae for G3. Addition of SCB in either feed or water produces superior results compared to control groups in terms of performance, productivity, biochemistry indices and intestinal integrity.

Introduction

The addition of probiotic yeast in the feed of domestic animals gains momentum as demands for reducing and banning antibiotic usage are increasing. Probiotic yeasts have been widely studied as a potential additive to overcome this burden in the last decade. One of the most studied yeast is *Saccharomyces cerevisiae boulardii* (SCB). They are not natural inhabitants of the intestines of poultry and other monogastric animals therefore they do not bind to the intestinal wall and remain active in the lumen (1). There are several ways of action in the organism and these include immunomodulation by activating the immune system through modification of cytokine function, metabolic effects by inhibition of toxins, improving the intestinal microflora, improving the feed digestibility, and lowering the cholesterol level, alteration of intestinal microbiota, and removal of oxygen thus increasing the colonization of viable anaerobic bacteria which leads to improved health and production parameters (2). This makes probiotics a valid alternative to antibiotics even in thermal stress conditions. Their multi-level modes of action translate into better performance of birds, more resistant birds, and safe animal protein products (3). Taking into consideration positive effects and modes of action authors across the globe have paid considerable attention to studying in more detail the beneficial effects of SCB in broilers. Broilers fed SCB diets had increased *Lactobacillus spp* colony-forming units and decreased *Clostridium spp* colony-forming units in the ceca. Moreover, the effects of SCB are not diminished by combination with other additives such as butyric acid on the contrary it shows a synergistic effect. The last is true when evaluating villus height and crypt depth (4). Besides probiotics other additives have been shown to improve the intestinal morphology (5) but still lack level of commercialization unlike bacterial- or fungal- origin probiotics. Local intestinal immunity is improved in broilers receiving SCB in feed. This is proven by an increase in the concentration of tumor necrosis factor-α, interleukin (IL)-10, transforming growth factor-β, and secretory IgA (6). Despite many proven positive effects of SCB, the influence on thyroid hormones and hormones regulating the metabolism of calcium and phosphorus is not conclusive and straightforward (7). Parallel, to the development and wider use of next-generation sequencing studies on chicken microbiota have increased, as well. The challenge of studying chicken microbiota is that chickens hatched in commercial environment have different microbiota than ones hatched in the natural environment due to lack of contact

with hen. Similarly, the way of acquiring the microbiota is different. Probiotics help to overcome this challenge but new species should be studied in the future (8). Addition of SCB results in the higher number of beneficial bacteria in the chicken microbiota and can help fight the pathogenic bacteria challenges, like Campylobacter jejuni by decreasing its abundance in the intestines (9). In our study, we investigated the effects of different doses of SCB in feed and in water as well, on production performance, hematology and biochemistry parameters, intestinal microflora, and intestinal microbiota in broilers.

Material and Methods

In this study, we performed two stages of field and laboratory investigations. Both stages were done on commercial broilers on the farm and lasted during the whole grow-out period, from day-old chicks to slaughter, i.e., day 42. The experimental groups were set up on the farm and kept under the same roof as other commercial broilers thus simulating real-life scenarios. The number of feed pans, drinkers, and nutritional requirements of the feed were according to the specifications and management guide. In the first stage, we evaluated the effects of different doses of feed addition of *Saccharomyces cerevisiae boulardii (SCB)* on multiple parameters of broiler health and productivity. In the second stage, we chose the most beneficial dose from the first stage and added the SCB both in feed and in water.

The first stage trial was done on four groups of 50 chicks, each group consisting of two replicates of 25 chicks, and in total 200 chicks. Group 1 didn't receive SCB in the feed (control group), group 2 received 150 g/1000 kg of SCB $1,5x10^{10}$ CFU/g, group 3 received 500 g/1000 kg of SCB $1,5x10^{10}$ CFU/g (0,05%) and group 4 received 1000 g/1000 kg of SCB $1,5x10^{10}$ CFU/g.

The second stage trial was done on three groups of 50 chicks, in total 150 chicks. Group 1 received 500 g/1000 kg of SCB $1,5x10^{10}$ CFU/g in feed, group 2 didn't receive SCB either in the feed or water (control group), and group 3 received 250 g/1000 l of SCB $1,5x10^{10}$ CFU/g in drinking water.

Productivity data was assessed by weekly measurements of the body weight, the feed conversion rate was estimated after the completion of each feeding phase (starter, grower, and finisher) and after the completion of the grow out, and the production efficiency factor was also calculated. In addition to the descriptive statistics, a comparison was made between the groups for the days when the measurements were performed. For that purpose, first, the distribution of the measured weights was analyzed with Shapiro - Wilk W test (criterion: $p > 0.05$) where the normal distribution was determined, and then through the Grubs test the possible presence of outliers was detected. After purifying the data, a one-way ANOVA test was conducted where the criterion for the statistically significant difference was an alpha criterion of $p < 0.05$, and the differences between the groups were determined by the Tukey HSD test.

Blood was collected at day 42 at the end of each stage and hematology and biochemistry indices were measured. For hematology, heterophile and lymphocytes percentage were determined as well as the heterophile to lymphocytes ratio. For biochemistry, several parameters were measured (albumins, globulins, total proteins, glucose, cholesterol, triglycerides, ALP, ALT, AST, urea, creatinine, calcium, and phosphorus). Descriptive statistics were performed on all data. Each parameter was statistically analyzed using the Kruskal-Wallis ANOVA test followed by Multiple comparisons of mean ranks using $p < 0.05$ as a criterion for significance.

After completion of the first stage, at the slaughter line, cecal and ileal content were collected and identification and enumeration of *Salmonella spp., Campylobacter spp., Clostridium spp.,* and *Lactic acid bacteria* was done. Descriptive statistics were made on the number of specific bacteria for each group at three levels: a) from all samples taken in the group; b) from a group of caecum specimens; c) from ileum specimens in a group. At the three levels, a comparison was made between groups to determine any differences in the number of bacteria. These assays were performed for lactic acid bacteria and Clostridium sp. because in the other bacteria the finding was the same in all groups. Comparative analysis between groups was performed using the Kruskal-Wallis ANOVA test using $p < 0.05$ as a criterion for significance.

After completion of the second stage, microbiome analysis of the fecal content was performed. For this purpose, bacterial taxonomic profiling was done on the V3-V4 region covering cca 445 bp of the 16S ribosomal RNA of four to five samples from each group. A set of high-quality reads was processed using minimum entropy decomposition. To assign taxonomic information to each operational taxonomic unit (OTU), DC-MEGABLAST alignments of cluster representative sequences to the sequence database were performed. A most specific taxonomic assignment for each OTU was then transferred from the set of best-matching reference sequences. Hereby, sequence identity of 70% across at least 80% of the representative sequence was a minimal requirement for considering reference sequences. Further processing of OTUs and taxonomic assignments were performed using the QIIME software package. Abundances of bacterial taxonomic units were normalized

using lineage-specific copy numbers of the relevant marker genes to improve estimates.

Results and Discussion

The use of SCB as a dietary supplement significantly increases body weight over a period of 42 days. Quantitatively, using SCB increased the broilers' body weight by 17% compared to the control group. The greatest effects of SCB on body weight are observed from 35 days of the age of the birds to the end of the growth out of 42 days, i.e., being closer to market age the differences in body weight increase. Up to 35 days of age, no statistically significant effects of SCB on body weight can be noted. Regarding the amount of yeast used, significant effects have been recorded with the use of 500g and 1000g yeast per 1000 kg of feed, more precisely, higher doses give significantly higher values of body weight. The FCR and EPEF values indicate a difference between the control and SCB-treated groups. Group 3 (500 g yeast / 1000 kg of food) had the best values for these two parameters.

Several preliminary conclusions can be made based on the raw data for the biochemistry parameters. Albumins in group 4 are significantly lower than those in group 1, and total proteins and globulins are significantly lower in groups 3 and 4 compared to groups 1 and 2. Glucose is significantly lower in group 4 than in group 1. Cholesterol and triglycerides are significantly lower in group 4 compared to all other groups. ALT is significantly higher in group 3 compared to other groups while AST is significantly higher in group 4 compared to other groups. Urea in group 4 is significantly lower than in groups 1 and 2 and, creatinine is lower in group 2 than in group 4. Calcium is significantly lower in groups 3 and 4 compared to groups 1 and 2 while phosphorus is significantly lower in group 3 than in group 1.

Results from the bacteriological analysis revealed an absence of *Salmonella spp.* in all groups which did not allow us to determine the possible influence of the SCB on this bacterium. The equal number of *Campylobacter spp*. in all samples from the groups regardless of treatment indicates the absence of the effect of the SCB on these bacteria at the applied concentrations. There is a declining trend in the number of bacteria of *Clostridium spp.* in groups with higher concentrations of yeast in the diet (groups 3 and 4). This trend is observed in samples taken from the caecum, which is not the case with ileum samples. However, no statistically significant difference was found between the groups and the effect of the SCB on this type of bacteria could not be confirmed. The number of *Lactic acid bacteria* is higher in the group with the highest concentration of yeast, especially in the caecum, and a similar tendency is observed in the ileum.

There were marked differences in the productivity data between the groups in the second trial. Group 1 had the lowest feed conversion ratio (1.49) and the highest production efficiency factor (448) while group 3 had the highest final body weight (2861 g) compared to other groups.

Microbiome profiling data identified taxonomic units in each sample from all three groups. Differences were found in the microbiome of samples from the three groups. Four samples from group 1 had the highest abundance of Bacillaceae 89,7%, Thermoactinomyces 99,0%, Bacillaceae 96,8%, and Thermoactinomyces 98,3%, respectively. Four samples from group 2 had the highest abundance of Bacillaceae 74,67%, Bacillaceae 99,9%, Thermobacillus 64,1%, and Brevibacillus 74,1%, respectively. In two of the samples, clostridial and streptococcal communities were also identified. Five samples from group 3 had the highest abundance of Bacillaceae 94,3%, Thermoactinomyces 63,6%, Bacillaceae 63,2%, Rickenellaceae 30,1%, and Thermoactinomyces 79,2%, respectively. In two of the samples, clostridial communities were also identified.

Conclusion

Higher doses of SCB in feed (500 g/1000 kg and 1000 g/ 1000 kg) produce better results. Administration of SCB in feed and water gave similar results in terms of productivity however the feed group had higher abundance of beneficial microflora and no pathogenic bacteria. Finally, the concentration of 500 g/1000 kg of *Saccharomyces cerevisiae boulardii* $1{,}5x10^{10}$ CFU/g in feed significantly improves broiler productivity, positively affects certain metabolic processes in the organism, and enhances the intestinal microbiota of the broilers.

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IS[10] Incubation, Maintenance and Feeding Management to Limit Meat Quality Defects

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Summary

For more than 20 years, meat quality has become a major issue for the broiler industry, due to the development of cuts and processed products. As in pork, defects related to the kinetics of post-mortem pH fall were first described, with cases of pale and exudative PSE-like meat. More recently, structural disorders, described as myopathies, have appeared, affecting the technological, sensory and nutritional properties of the meat. Much work has been done to understand the etiology of these defects and their links with other production traits. This research, still in progress, is necessary to help the poultry sector to implement efficient strategies against meat defects, both through genetic improvement and adaptation of breeding conditions.

Introduction

Description of the defects and impacts : Poultry has become the first meat produced in the world since 2017 and is expected to account for almost half of the total growth in meat production in the coming decade. Several factors have contributed to the success of poultry meat, such as its relatively low price, healthy image of the product, considered as lean and rich in protein, absence of religious barriers and convenience of the product. Indeed, during the last decades, the poultry sector has undergone a major transformation in order to adapt to changes in the consumption habits, supplying most chicken meat as cut-up portions or elaborated products, and less as whole carcasses. This expansion of poultry production, initiated in the 1950s, has been linked to the emergence of industrial poultry production based on the use of highly productive genetic strains and the optimization of breeding and sanitary conditions as well as inputs (especially nutrition). Comparing a commercial line with two unselected lines maintained since 1957 and 1978 at the University of Alberta, Zuidhof et al. [1] showed that over a period of almost 50 years, broiler industry has been able to reduce the amount of feed required to produce chicken meat by one-half. In the same time, consequences on the histological and metabolic characteristics of the muscles of the selected birds have been reported, such as a hypertrophy of fibers and a decrease in glycogen reserves and vascularization [2,3,4], that may have pave the way for further muscle disorders.

The first studies on meat quality focused on describing and understanding the origin of PSE-like defects, referring to the problem described in pork. This meat is characterized by pale color and higher water loss during storage and cooking, making it tough and not very juicy once cooked [5]. Due to the low water holding capacity, PSE-like meat also has low processing capacity. The term PSE-like is generic and covers two types of defects: PSE meat showing a very rapid drop in post-mortem pH (with a pH at 15 minutes post-mortem most often below 6), or acidic meat with an ultimate pH (measured 24h post-mortem) below 5.7. Most often only the ultimate pH is measured the day after slaughter. Unlike acidic or PSE meats, DFD meats are characterized by a dark color and a high ultimate pH that results in increased water-holding capacity. As a result, these meats are more susceptible to microbial development and to off-odour development that impair the product shelf-life [6]. In a field study conducted in France twelve years ago, we looked at the variability of the ultimate pH in different production systems. Alternative production systems, using slow-growing strains, were more concerned by acidic meat problems (about 50% of the fillets with a pHu lower than 5.7 for Label Rouge chicken) than standard production systems (less than 20%). Cases of DFD meat (pHu greater than 6.2) were only found in standard chicken or heavy chicken production, but at this time the frequency remained below 5% [7].

More recently, the poultry sector became concerned with an increase in the frequency of new muscular disorders, qualified as myopathies, which affect the technological, sensory, and nutritional properties of the meat but also the consumer acceptance. In a recent comprehensive review, Petracci et al. [8] did a state of knowledge of the putative causes and consequences of “white striping” (WS), “wooden breast” (WB) and “spaghetti” muscles (SM). Briefly, WB is characterized by hardened and pale areas and, depending on the severity of the condition, presence of haemorrhages and exudate on the muscle surface. WS is characterized by visible white lines, which are parallel to the muscle fibres and composed of adipose tissue. In case of SM defect, a loss of integrity of the muscle is observed, with a tendency towards separation of the fibres bundles composing

the muscle tissue. WS and WB, which can be observed together or independently in the breast muscle, share common histological characteristics such as degeneration and regeneration of muscle fibres together with an increased deposition of adipose tissue (lipidosis) and of connective tissue (fibrosis). Studies on the molecular processes involved also show alterations in carbohydrate metabolism, protein synthesis and a significant increase in oxidative stress, associated with signs of inflammation and hypoxia.

Except for tenderness measurements, all studies on the technological quality of fillets agree to show that breast meat with WS and/or WB have a higher exudate and cooking losses, a lower assimilation of the marinade, as well as a lower technological yield than normal meat. Regarding the pHu of fillets with WS or WB, it is generally higher (up to 0.2 to 0.4 pH unit) than that of normal fillets, while their Water Holding Capacity (WHC) is much lower. The depletion of myofibrillar and sarcoplasmic proteins and the accumulation of interstitial materials such as water, collagen, and proteoglycans observed in these myopathies, would explain the low WHC of the affected meat [9]. It should be noted that, compared to WS, the presence of WB or both defects within the same fillet markedly enhances muscle WHC degradation.

Regarding the prevalence of the defects, for WS, the most common of these myopathies, average rates of 50% were reported (according to studies in several European countries and in Brazil), with the most severe cases accounting for 20% to 30% of the breast muscles [8]. These results are consistent with a survey conducted in France in 2017 in several industrial slaughterhouses on 123 batches of certified, standard and heavy chickens [10]. It revealed that 66% of the samples exhibited WS defect, including 15% to a severe degree. Moreover, 53% of the samples were classified as WB (including 22% to a severe degree) and 11% as spaghetti muscle. The results obtained in Italy over the same period (between 2017 and 2018 on 16.000 fillets) showed similar figures, with 42% of samples showing moderate WB and 18% severe WB, and 20% of SM muscles [8]. Even if the figures may vary according to the scoring grids applied and the characteristics of the flocks studied, they show the seriousness of the problem and the difficulties of the poultry meat sector in managing it.

Factors of variation of breast muscle myopathies (BMM)

Given the rapid increase in the frequency and severity of BMM in slaughterhouses, a large number of studies have been conducted in recent years to better understand the main factors that contribute to their development and to find out strategies that could reduce their frequency and severity. The studies have considered factors related to the intrinsic characteristics of the animals but production factors such as nutrition.

Growth traits and genetics : It has been widely described that the incidence of WS and WB defects increases as animal performance improves (i.e. increased growth rate, higher breast weight and yield at slaughter). Thus, several studies have shown an effect of sex (males being more affected than females), age and weight of animals at slaughter and genotype. In this sense, Lorenzi et al. [11] reported that high-breast-yield hybrids exhibited a higher incidence of both moderate and severe WS compared with standard-breast-yield birds. They observed similar results when comparing birds with high slaughter weights (between 3.0 and 4.2 kg) to birds with medium slaughter weight (between 2.2 and 3.0 kg). In the literature, the involvement of genetics in the appearance of WS and WB defects is still subject to debate, but there were clear variations between pure lines, more or less selected on growth and muscle yield. In the study by Bailey et al. [12] including two pure lines, incidence of the different kinds of myopathies (WS, WB and deep pectoral myopathy) was much higher in the high-yield line than in the moderate-yield line, showing the role, even indirect, of muscle development. In the study by Alnahhas et al. [13] in two lines of standard chickens diverging for breast meat ultimate pH, a strong positive genetic correlation (0.68) was found between the susceptibility to WS and breast yield (and more specifically with the percentage of pectoralis major).

Nutritional factors : Because of its impact on growth and body composition, diet is obviously an important factor to consider in order to control the appearance of defects. Numerous studies have been carried out, that we will not be able to detail here. In general, it appears that the impact of a reduction in feed intake or nutrient density on the frequency of defects is mitigated. When positive effects are observed, they are most often accompanied by a decrease in growth and muscle development. Studies and field trials suggest that it is harmful to apply this reduction in the early phase of life (see Aviagen [14] report for more details), possibly because of the impact on the proliferation of satellite cells that contribute to muscle repair. On the other hand, various supplementation treatments with anticoccidials, antioxidants (vitamins E, C, selenium) or optimized pre-starter feed did not show a robust effect on the incidence of WS or WB defects. Other promising strategies have attempted to compensate for the lack of energy observed in BMM (including a decrease in muscle glycogen levels) by adding guanidinoacetic acid (GAA). This is a metabolic precursor of creatine and phosphocreatine, which are used by muscle cells to produce ATP. In 51-day-old broilers, dietary supplementation of GAA (600 g/ton) has reduced the severity of WB (mean values 2.33 versus 2.76 on a 4-point scale) and the ultimate pH of the meat, while slightly improving body weight and FCR [15].

Search for Solutions

The better knowledge of the etiology of the defects allows to orientate the search for solutions, which will undoubtedly have to be combined to reduce the frequency of these multifactorial defects. In this last paragraph, we will mention possible solutions related to genetic selection or breeding conditions.

Because of their impact on the intrinsic qualities of the meat but also on the image of the product, the reduction of breast muscle myopathies has certainly become one of the priorities of breeding companies. In order to meet this objective, new crosses can be produced to better respond to market expectations. The selection objectives in the pure lines that are used to produce these crosses must also evolve by finding the good combination between growth and muscle integrity and meat quality.

Regarding the first strategy, we recently evaluated performance and meat quality of six strains with different growth potential (Ross 308, Redbro, Rustic Gold, Ranger Classic, JA787, and JA757) reared at conventional or reduced density (39 or 30 kg/m²). Animals had access to natural light and enrichments and were slaughtered between 1.8 and 2.2 kg. Redbro and Rustic Gold strains are new alternatives with improved growth rate or meat yield compared to Ranger Classic and JA787, but still below Ross 308 performances. Compared to Ross 308, growth rate was reduced by 40% for the certified strain (JA757), and by 24 to 32% for the other strains. In the same time, a significant increase in feed conversion ratio was observed (8 to 35% according to the strain), and in ready-to-cook and breast yields. Unlike strain, density had no effect on performance or meat quality measurements. The strain had a significant effect on the breast (and thigh) meat ultimate pH, increased growth rate and breast yield being associated with less acidity (highest pHu in Ross 308 and lowest in JA757). In the same time, compared to Ross 308, total water loss (during storage and thawing/cooking) was 16% to 30% lower in the alternative strains. This was likely due to the presence of white striping and wooden breast defects observed in the fastest growing genotype (12% and 24% in the Ross 308 fillets, respectively) and known to strongly alter the water retention capacity of the meat. Of course, this improvement in meat quality was achieved at the cost of reduced growth and yield criteria in the alternative strains, but future genetic solutions could allow reducing the gap between productivity and meat quality.

For pure lines, the possibilities of selection on a trait depend both on its heritability (the part of phenotypic variance explained by genetics) and on its genetic correlations with the other traits of interest. To date there are very few published studies on the genetic parameters of muscle myopathies in broilers. The first one, published by Bailey et al. [12], included two types of pure lines (one high-yielding line, and one moderate-yielding line) and a significant number of measurements over 6 generations of selection. WS appeared much more frequent (14.5 to 49.6%) than WB (0.16 to 3.19%) and Deep Pectoral Myopathy (1.9 to 2.3%) whatever the line. The estimated genetic correlations between BMM and body weight or breast yield (BRY) were modest (-0.08 to 0.25) and overall more significant in the moderate yielding line than in the high yielding line. The progress to be expected from genetic selection to reduce susceptibility to muscular dystrophy was tested experimentally in the study by Bailey et al. [16] on lines contributing to the Ross 708 cross. In pure lines, future reproducers are selected on the basis of their predicted genetic values, that the breeder seeks to reduce for BMM (especially WB) and increase for production traits (especially BRY). In order to estimate the genetic progress obtained by this type of selection, offspring of Great Grand Parents (GGPs) were compared to terminal hybrids. The generational difference between the two broiler groups represents 2 years of genetic progress. Performance varied between the batches produced but the study reported a relative decrease of 18.4% in the incidence of WB and a relative increase of 1.02 in BRY. As illustrated by this study, selection is one of the long-term strategies for reducing the BMM while preserving progress on production traits. Since the performance of terminal hybrids not only depend on the genetic potential of pure lines but also on less predictable effects such as heterosis or environmental variations, it will be important to regularly assess the progress achieved on farm.

Muscle myopathies have been accompanied by several anatomical, histological and metabolic modifications, which can also suggest some indicators or biomarkers of interest for genetic selection. Thanks to a divergent selection for the breast meat ultimate pH, a proxy for muscle glycogen reserves, it was observed that the higher glycogen content obtained in the pHu- line was associated to a lower frequency and severity of White Striping defect [13]. In the pHu+ line, decreased muscle vascularity and glycogen content [17], coupled with alternative pathways of energy production through protein catabolism and lipid oxidation as well as oxidative stress [18,19], created favorable conditions for the development of the WS myopathy. In these pHu lines, WS is rather highly heritable (0.41 on a three points scale) and significantly correlated with intramuscular fat content (r_g>0.50). The latter measurement, easily measured by Near Infrared Spectroscopy from muscle samples, could be a valuable indirect indicator of WS defect, by allowing an accurate evaluation of the level of lipidosis. The numerous omics analyses have also made it possible to identify genes whose expression in the muscle varies with the presence of the defect. For example, a huge overexpression (x12) of myosin heavy chain 15 (MYH15) was observed in fast-growing chickens

affected by both severe forms of WS and WB compared to a control group visually free of the defect. MYH15 is transiently expressed in embryonic chick skeletal muscles and then re-expressed during muscle regeneration. Conversely, the expression levels of adult (MYH1E) and neonatal (MYH1F) myosin heavy chain isoforms were lower in the affected group than in the control groups [20]. Recent study has shown that histological technique based on fluorescence intensity measurement can be used to quantify gene markers related to muscle remodeling and regeneration such as FN1, NCAM, and MYH15 and to refine the diagnosis of WS and WB [21].

Of course, all these muscular phenotypes (ultimate pH or Glycolytic Potential, gene expressions, histological analyses) require sacrificing the birds and imply a sib-selection, as for the visual (subjective) scoring of the defects on deboned muscles. It would therefore be very interesting, especially for selection, to have new techniques - physical, imaging or biological - usable on live animal. Regarding the blood parameters, it has been shown that severe forms of WS is associated to elevated serum levels of creatine kinase, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase [22]. Recently, Kong et al. [23] reported good phenotypic correlations between the compression force of breast deboned muscle and WB score (r=0.72) as well as with serum Creatine Kinase activity (r=0.61), a marker of muscle damage. Thanks to an untargeted metabolomics analysis, Lake et al. [24] reported a strong association between 3-methylhistidine, often used as an index of myofibrillar breakdown in skeletal muscle, and wooden breast and white striping. This metabolite showed a huge heritability ($h^2 = 0.90 \pm 0.18$) in their population, supporting that it could respond to selection. Interest of such biological markers in the selection programs have to be further investigated.

As pointed out in the study by Papah et al. [25], the major cellular and molecular perturbations seen in BMM are already present by 3 weeks of age (even before the disease is clinically evident by palpation). This suggests that early mitigation strategies, focused on the first days of life or even incubation, are likely to be prioritized. As explained in the review by Oviedo-Rondón et al. [26] on the role of incubation conditions, myopathies could be induced by stressful conditions during the last phase of incubation, due to high temperatures or low oxygen concentrations. These incubation conditions have thus to be optimized, by taking into account the chick genetics which can impact its development and metabolism as well as the eggshell characteristics.

Conclusion

The emergence of muscle defects over the last decade is a sign that biological limits have been reached in some animals. Strategies for mitigating these defects, particularly through genetics, are based on the search for the best compromise between performance, robustness and product quality. The accumulation of knowledge on the etiology of defects and the development of diagnostic tools will be an asset to develop new genetic and breeding solutions.

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OP26 The Effect of Male Broiler Parent Body Weight Differences during the Rearing Period on Progeny Broiler Performance

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Abstract

According to body weight (BW) 300 roosters were determined in light, standard and heavy pens at the 6th week of rearing period of male broiler breeders with Ross 308 genotype. At the 18th week of rearing, those with the lightest (LL) and standard (LS) weights in the light pen, as standard (SS) in the standard pen, and standard (HS) and the heaviest (HH) males in the heavy pen were selected for the production period. The study was carried out to examine the broiler performance results of artificial insemination of these roosters with standard weight hens at different weeks of age (24-Young, 35-Prime and 48-Old). When broiler performance characteristics were examined, it was determined that the HH group, which had the highest average BW in all studies, was ahead by a significant margin ($p<0.05$). Although the order of the other groups varies according to the age of the sire, the LS group had the lowest average in general ($p<0.05$). HH group broilers had an advantage in terms of FCR in addition to BW ($p<0.05$). According to the European Production Efficiency Index (EPEI), HH group broilers reached the highest values ($p<0.05$). As a result; It has been determined that selection cannot be made according to the weight of only the 6th week roosters and that being at a standard weight adversely affects broiler performance. It is suggested that HH group males can be preferred for heavier and more economical broiler production.

Introduction

Body weight gain has been the first and most important parameter in the breeding of meat type chickens. It will maintain its importance in the future as well [1, 2]. After evaluating the performance on the amount of meat obtained from long-term broilers at slaughter age, it was understood that the amount of meat obtained per breeder in the 1990s should also be used in the evaluation [3]. Unlike layers, body weight control and uniformity are of greater significant in the rearing of broiler parents. A male broiler breeder can produce up to 1500-2000 chicks in an egg production period of about 40 weeks. In broilers with high liveability, 2.3-2.8 kg slaughter weight and 1.5-1.7 feed conversion ratios were achieved in 5-6 weeks [4, 5]. Each male breeder is of great importance in order to obtain the highest benefit from the meat lines with superior genetic structure, which are created by breeding companies with very difficult processes [6]. Breeding companies place great emphasis on selecting the heaviest males in their sire line populations for to obtain heavier broilers. Parent-stock breeders rarely use this information because they think the breeding companies have completed this selection. Although breeding companies have made the selection, there is still enough genetic variation among these selected animals to allow the selection of heavier males [7]. This study was carried out to determine the effect of 6 and 18 weeks body weights of rearing period of broiler male parents on progeny broiler performance results.

Material & Method

Selection of Male Parents : The male parents of the experiment was obtained from the broiler parent rearing house of Erpiliç firm (Turkey). At the 6th week of growing period, all birds were individually weighed. The wing tags of 300 males were determined from the light (100 males), standard (100 males) and heavy (100 males) pens, with an average of approximately 850, 1150 and 1450 g, respectively. These birds were also marked with blue paint on their backs and continued to be reared in their own pens throughout the rearing period. Thus, as a result of the body weight values at the 6th and 18th weeks, the cockerels were divided into 5 groups as Light Light (LL), Light Standard (LS), Standard Standard (SS), Heavy Standard (HS) and Heavy Heavy (HH). A total of 25 cockerels, 5 individuals from each group, were determined. Average weights in the groups were determined as 2230, 2715, 2740, 2760 and 3260 g, respectively. The cage dimensions were 47x50x5 cm and had been adjusted so that 1 nipple drinker and 1 bowl feeder per male.

Selection of Female Parents : From the parent flock of the 45-week-old Ross 308 genotype reared by the same integrated company, 220 females, whose BW was in the range of 3800-3900 g and which were determined able to lay eggs after

physical controls, were selected and transferred to Poultry House of Ankara University. One week after the hens came to the experimental house, they were individually weighed again and their wing tags were attached. In this weighing, BW means were fond to be 3919 g, and females were determined so that the BW means were equal for each male group. The male groups to which the females belong were coded in front of their cages. Individual cages are 55x45x40 in size and 1 nipple and 1 feeder per female were used so that they would no eat each other's feed.

Egg Production Period : In order to have females and males belonging to each group in each part of the house, the males and females were randomly distributed to the cages on the floors and rows. There are a total of 25 males and 220 females individual cages in the house. The 8 h daily lighting period applied to the males was continued for 1 more week after they were transferred to the Poultry House. The first light stimulation for males began at 21 weeks of age. The lighting time at the beginning of this week had been increased to 10 h and to 12 h at the end. When the hens arrive at the house at 22 weeks of male age, the lighting is fixed at 14 h. As much as possible, homogeneous lighting was provided in the house with an average of 60-80 lux at bird levels. Feeding of the parents was done once a day between 8-9 am. Feed containing 2800 kcal/kg ME, 14% CP and 3.2% Ca, prepared in granule form, obtained from a commercial company.

Determination of Broiler Performance **:** According to the age groups of the sires, a total of 3 broiler studies were carried out young, prime and old. In broiler experiments, chicks hatched between 490-500 h of incubation were used to minimize the effect of hatching time. The chicks were weighed by tags individually according to the sire groups after sex determination and reared. Then, the weighed chicks were reared in the Broiler Research House with equal number of male and female chicks per pen (Table 1).

Table 1. Number of pens and chicks used in broiler experiments

Experiments	Pen per group	Total Pen	Broiler per Pen	Broiler per Group	Total Broiler
1	7	35	14	98	490
2	9	45	14	126	630
3	5	25	16	80	400

During the rearing period, 24 h of continuous lighting was provided in the house with a light intensity of 50-60 lux. Feed and water were given ad-libitum for broilers during all rearing periods. Starter (3,000 kcal ME/ kg and 23.5% CP) and grower (3,200 kcal ME/kg and 22.0% CP) diets were fed for 0 to 10 and 11 to 28 d, respectively. A finisher (3,300 kcal ME/kg and 20.0% CP) diet was fed from 29 to 35 d. Three nipple drinker, a chick feeder for the first 7 d, and a hanging feeder after 7th d of weighing were used in each pens. In the first 4 d of trials, graph paper was laid on 60-70% of the litter (with the chick feeder in the middle of the paper) and feed was sprinkled on this area, so that the chicks could reach the feed more easily. The chicks were weighed with an accuracy of 0.01 g at the time of placing in the pen (510-515 h of incubation). On the 7, 14, 21, 28 and 35 d of rearing were determined individual BW, and were calculated per pen the average feed consumption and the feed conversion ratios (FCR). In these periods, scales with a precision of 2 g were used.

Statistical Analyses : In the trials, body weight (BW), mortality rate (MR), feed conversion ratio (FCR) and European production efficiency index (EPEI) were calculated. While calculating FCR, dead broiler weights were also taken into account. BW weighings were carried out individually and as well as broiler performance criteria (BW, FCR and MR) were calculated EPEI, where these values were evaluated together. In the calculation of MR, FCR and EPEI, the value of each pen was evluated as an replication. Care was taken to prepare the experiments in a randomized design. MS Excel computer program was used to organize and store the data. All statistical analyses were performed with the relevant procedures of the SPSS 20.0 program [8]. DUNCAN multiple comparison test was used to determine the different groups. The statements of statistical significance were based on $P \leq 0.05$, unless otherwise indicated.

Results

Body Weight (BW)

Experiment 1: The heaviest chick weight started in the HH group, but this group was the lightest in the first week ($p<0.05$). In the following week, weight gain accelerated and reached the highest weight in the 3rd week. When the weights of the 4th and 5th weeks were examined, it was seen that the HH and HS group have the highest, SS and LL moderate and the lowest BW were in the LS group ($p<0.05$).

Experiment 2: LL and HH group chicks have a higher weight compared to the other groups in terms of weight on the house

placement day. HS and SS groups followed the BB group in the first week of weighing, while the LS and LL groups remained at the lowest mean. LL group reached the same weight as the HS and SS groups in terms of body weight gain in the periods after the first week. While the highest BW average was calculated in the hh group, the lowest was determined in the LS group ($p<0.05$). In experiment 2, similar to the first, the LS group had the lowest mean, while the LL, SS and HS groups similarly behind HH group.

Experiment 3: In terms of house placement time weight, HH and SS had the highest mean weight, followed by LS and HS, and the LL group had the lowest weight ($p<0.05$). Although the differences in the first week were not significant, when we looked at the last week's means (Table 2), the HH group differed by 90 g from the close group and the averages of the other groups were close to each other ($p<0.05$).

Table 2. Average 35th d BW of Broilers

BW Groups	Sire Ages (Week) / Experiments			Average
	Young (24)	Prime (35)	Old (48)	
	1	2	3	
	---------- g ----------			
Light Light (LL)	2465[b]	2126[b]	2395[b]	2320[b]
Light Standard (LS)	2397[c]	2029[c]	2432[b]	2250[c]
Standard Standard (SS)	2492[b]	2128[b]	2409[b]	2320[b]
Heavy Standart (HS)	2562[a]	2125[b]	2386[b]	2319[b]
Heavy Heavy (HH)	2574[a]	2256[a]	2523[a]	2431[a]
SEM	*13.75*	*15.73*	*23.06*	*18.65*

a,b: According to Duncan's test, there is a difference between means with different letters in the same column ($p<0.05$)

In terms of the 35th d weights of broilers (Table 2), the LS group had the lowest average and LL, SS and HS were close to each other, while the HH group reached the highest average in all periods. BW averages of the last week, HH group differed by 88, 129, 92 g, respectively, and 111 g when looking at the general averages of these 3 experiments.

Feed Conversion Ratio (FCR)

Experiment 1: There were differences in the numerically from the 14th d, and the SS group, which was high until the last week, showed improvement as of the last week, while the HS group was found to be in the worst average ($p<0.05$).

Experiment 2: While the differences between the groups in the second week were not observed on the 21st and 28th d, the HS group reached the highest FCR and the HH and LL groups reached the best FCR in 0-35 d period ($p<0.05$).

Experiment 3: Differences between trial groups were not found significant ($p>0.05$). It is seen that the HH group was good not only BW but also FCR (Table 3).

Table 3. Average 0-35th d FCR in Broilers

BW Groups	Sire Ages (Week) / Experiments			Average
	Young (24)	Prime (35)	Old (48)	
	1	2	3	
	---------- g/g ----------			
Light Light (LL)	1.459[ab]	1.563[bc]	1.510	1.511[b]
Light Standard (LS)	1.472[ab]	1.572[bc]	1.486	1.510[b]
Standard Standard (SS)	1.454[ab]	1.579[ab]	1.500	1.511[b]
Heavy Standart (HS)	1.486[a]	1.611[a]	1.513	1.537[a]
Heavy Heavy (HH)	1.438[b]	1.540[c]	1.510	1.496[c]
SEM	*0.013*	*0.012*	*0.016*	*0.008*

a,b: According to Duncan's test, there is a difference between means with different letters in the same column ($p<0.05$)

Mortality Rate (MR)

Experiment 1: Although the LS, SS and HS groups had a higher mean compared to the LL and HH groups, the increase in MR was higher in the LS and HS groups, which reached the srtandart weight at the 18th week, compard to the SS group ($p<0.05$).

Experiment 2: Although the MR were higher in the SS group compared to the other groups, the differences were significant only at the 2nd week ($p<0.05$).

Experiment 3: Although the MR in the HS group were higher than the other groups from the first week, this difference was insignificant in the last period (Table 4).

Table 4. Average 0-35th d Mortality in Broilers

BW Groups	Sire Ages (Week) / Experiments			Average
	Young (24)	Prime (35)	Old (48)	
	1	2	3	
	---------- % ----------			
Light Light (LL)	1.02[b]	2.38	1.25	1.71
Light Standard (LS)	5.10[a]	1.58	1.25	3.05
Standard Standard (SS)	4.08[ab]	4.76	1.25	3.88
Heavy Standart (HS)	6.12[a]	3.17	5.00	5.30
Heavy Heavy (HH)	0.00[b]	2.38	3.75	2.25
SEM	*1.65*	*1.35*	*1.35*	*1.306*

a,b: According to Duncan's test, there is a difference between means with different letters in the same column ($p<0.05$)

European Production Efficiency Index (EPEI)

In the 3 broiler study, the average EPEI values of LL, LS, SS, HS and HH groups were found to be 434, 423, 429, 422 and 460, respectively (Table 5). In terms of EPEI, HH group had the highest and LS group had the lowest index in the first experiment ($p<0.05$). In the second trial, HH was again the highest mean, while the other groups were similar. In the last trial, the LS group had the highest mean, followed by the HH, SS and LL groups, and the last group was HS ($p<0.05$). Looking at the general average, it was determined that while the HH group reached the highest EPEI, the other groups had similar averages ($p<0.05$).

When broiler performance characteristics were examined, it was calculated that the HH group, which had the highest average in terms of BW on the 35th d in all studies, was ahead of the closest groups by a significant difference about 110 g on the general average ($p<0.05$). Although the order of the other groups varies according to the age periods, the LS group had the lowest average in general ($p<0.05$). HH group broilers also provided an advantage in terms of FCR due to the low feed consumption ratio compared to body weight gain ($p<0.05$).

The FCR mean of the LL group was also higher than the other groups ($p<0.05$). According to the EPEI averages in which performance criteria such as BW, FCR and liveability were evaluated together, the HH group broilers reached the highest values, while the LL group was in the second place and the LS, SS and HS groups took the last place ($p<0.05$).

Table 5. Average 35th d EPEI in Broilers

BW Groups	Sire Ages (Week) / Experiments			Average
	Young (24)	Prime (35)	Old (48)	
	1	2	3	
	---------- EPEI ----------			
Light Light (LL)	476[ab]	382[b]	447[ab]	434[b]
Light Standard (LS)	445[d]	363[b]	464[a]	423[b]
Standard Standard (SS)	470[cd]	363[b]	453[ab]	429[b]
Heavy Standart (HS)	472[bc]	363[b]	425[b]	422[b]
Heavy Heavy (HH)	513[a]	409[a]	449[ab]	460[a]
SEM	*8.93*	*6.65*	*9.12*	*4.79*

a,b: According to Duncan's test, there is a difference between means with different letters in the same column ($p<0.05$)

Discussion and Conclusion

Over the years, it has been reported that the share of genetics in the improvement in broiler performance traits is 85% [9, 10]. Breast meat ratio, FCR and slughter weight come to the fore compared to reproductive traits in terms of contributing to the profit increase rate of integrated companies [6]. However, the most fundamental factor affecting this profit increase is the broiler meat production per parent [11]. Currently, studies on the performance of broiler progeny of male parent body weights have generalized to heavy males and others [7, 12, 13]. Our study differs from other studies due to the comparison of groups consisting of both light and heavy group as a result of standart weighting during the rearing period. Contrary to the popular belief about standart weights, the HH group comes to the fore as more and economical meat production is targeted. However, in order to make a clearer interpretation, studies on the economic analysis of the yield parameters obtained from all group cocks are required. As a result, it has been determined that the parent stocks (PS) cannot be selected according to the weight of cocks for only 6th weeks, the performance of the offspring decreases when they reach the standart weight at 18 weeks when they are at heavy body weight this week, and the performance of their progeny broilers is the lowest when the light cocks at 6th weeks reach the standart weight at 18th weeks. In summary, it was determined that the body weight changes were not good after the 6th weeks and the broiler performance of the offspring of the heavyweights at the 6th week and heavy at the 18th week was the highest. Considering the EPEI, in which FCR and MR are also included in the formula in addition to BW, it is suggested that HH group cocks should be preferred for heavier and more economical broiler production.

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OP[27] Genetic Variability of Laying, Egg Quality and Meat Quality Traits in Broiler Lines With Different Muscle Glycogen Levels

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Abstract

This study aimed to estimate the heritability of reproductive performances and egg quality characteristics and their genetic correlations with growth and meat quality in broiler lines with different muscle glycogen levels. For this study, we considered the pHu+ and pHu- lines divergently selected for the breast meat ultimate pH (pHu), a proxy for the muscle glycogen reserves. Regarding reproduction traits, age at first egg (AFE) as well as egg production (EP) and broken egg production (BRKEP) from AFE to 38 weeks were individually determined in the pHu breeders, from generation (G) 2 to 14. For egg quality traits, egg weight (EW) and egg shell percentage (ESP) were measured in all eggs for four consecutive days, from 25 to 32 weeks in G13 and 25 to 42 weeks in G14. Breeder body weight (BREBW, g) in females, broiler body weight (BROBW, in g) and the pHu of the *Pectoralis* muscle (BRpHu) of female and male broilers was determined individually at 6 weeks. Heritability (h^2) for each trait and genetic correlations (r_g) between traits were estimated using VCE 6.0.2 software with the restricted maximum likelihood (REML) method in a single-trait and bivariate linear animal model, respectively. After 14 generations of divergent selection, the main selection criterion, BRpHu, moved from its original value (5.88) in the base population to reach 6.20 in the pHu+ line and 5.56 in the pHu- line. Significant differences between the two lines were observed from G3 for BRpHu, while correlated responses appeared for EP and BRKEP from G5. The pHu- line had an average of 5 more eggs produced and 1 fewer broken egg per hen compared to the pHu+ line. The latter line also exhibited lower BREBW from G5 and lower BROBW from G7. No significant Line x Generation effect was found for AFE whose mean values were 183.5 and 186.5 days in the pHu- and pHu+ lines, respectively ($P < 0.001$). In the two last generations (G13 and G14), EW was higher in the pHu+ line than in the pHu- line (58.0 vs 55.3 g, P<0.001) while ESP was lower (8.99% vs 9.56%, $P < 0.001$).

The h^2 estimates were similar in the two lines and were moderate to high for the different traits (0.34-0.50 and 0.38-0.56 for the pHu- and pHu+ lines, respectively), except for BRKEP (0.07 and 0.16 for pHu- and pHu+ line, respectively). Strong negative genetic correlations were found between AFE and EP whatever the line. In the pHu- line only, a moderate but significant positive genetic correlation was evidenced between BRpHu and AFE (0.32), while the selection criteria was negatively correlated with EP (-0.20). In both genetic lines, AFE was moderately negatively correlated with ESP (r_g of -0.36 to -0.42) and positively with EW (r_g of 0.37 to 0.43). Quite high negative genetic correlations were found between BRKEP and ESP. BRKEP was also moderately negatively correlated with total egg production (-0.33 and -0.20 in the pHu+ line and pHu- line, respectively). A moderate but positive genetic correlation was found between BREBW and EW (0,29) and between BROBW and BRKEP (0.22) in the pHu+ line, while no significant correlation was found with any of the laying or egg traits in the pHu- line.

The current study showed that genetic selection for pHu diverged the lines phenotypically not only for meat characteristics but also reproduction and egg quality characteristics. Selection for higher muscle energy reserves in the broilers of the pHu- line led to positive correlated responses on reproductive functions (earlier AFE and higher EP) in their breeders, suggesting a better biological efficiency of this genotype. The poor egg-laying performances in female breeders of the pHu+ line could be due to a mismatch between the metabolic needs of these animals and their diet (in quantity and/or quality).

Introduction

Genetic selection for fast-growth, breast yield and feed efficiency, which are highly heritable and economically important

traits in modern broilers, has created great genetic potential for meat productivity over decades [1]. Unfortunately, selection for growth traits has changed the anatomical, morphological and physiological status of broilers against their health, welfare [2], meat quality [3] and reproductive fitness in broiler breeders [4]. Selection for growth and breast traits caused a decrease in the muscle glycogen content, which is highly negatively correlated with the breast meat ultimate pH at the genetic level [3]. Genetic studies have shown that ultimate pH is a good selection trait for the improvement of meat color, water holding capacity, and texture in breast meat, as it has high heritability and is genetically correlated with other meat quality traits [3]. Since 2009, two divergently selected broiler lines (pHu+ and pHu-) were created by selection for low and high breast muscle pH (Pectoralis major pHu) in order to understand better the physiological and genetic mechanisms of meat quality traits in broiler chickens. These lines are also a unique genetic material that reflects low and high muscle glycogen levels [5,6], and the amount of glycogen reserved in Pectoralis major muscles was average 25% lower in the pHu+ line than in pHu- [7]. The glycogen reserves in the muscle represent a substantial energy source for the bird. Egg production and quality depend on the physiological and maternal fitness of female. The metabolic status of the hen and structure of the egg affect the embryonic development and post-hatch parameters of the chick [8]. The relationships between growth and reproductive traits have been studied extensively from past to present [2,3,9]. However, to the best of our knowledge, this is the first paper on the genetic associations between meat quality and reproductive performance, and egg quality traits in poultry. The objectives of this study were to estimate the heritability and genetic correlations between some growth and meat quality in broilers with different muscle glycogen levels and reproductive and egg quality characteristics in their breeders.

Material and Methods

The present study was conducted in agreement with the French National Regulation for human care and use of animals for research purposes. Animals were reared at the PEAT INRAE Poultry Experimental Facility (2018, https://doi.org/10.15454/1.5572326250887292E12) registered by the French Ministry of Agriculture (INRAE, Centre Val de Loire, Nouzilly, France). Data were collected from two experimental broiler lines with low (pHu-) and high breast meat ultimate pH (pHu+), divergently selected over 14 generations based on the estimated genetic value for breast meat ultimate pH (pHu) measured on 6 weeks-old broilers as selection criteria. For reproductive traits, data were obtained from 57295 eggs from 886 pHu- line hens and 49596 eggs from 854 pHu+ line hens between age at first egg and 38 weeks from G2 to G14. For egg quality traits, 2031 eggs from 157 pHu- line females and 1486 eggs from 147 pHu+ line females were used. For broiler characteristics, data were collected from 2451 to 2608 broilers in the base population, and from 1954 to 3565 broilers in the pHu- line and 1866 to 3455 broilers in the pHu+ line from G1 to G14 of the selection.

Similar procedures were used for all birds in each generation in terms of hatching, management, feeding, vaccination and sanitary practices. To reduce the effects of the environment, birds from the two lines (pHu+ and pHu-) were reared together in a standard closed broiler house equipped with a dynamic ventilation system, gas heaters, automatic pan feeders, and nipple drinkers at PEAT INRAE Poultry Experimental Facility.

The reproductive traits were recorded individually for both lines from G2 to G14. Age at first egg (AFE, age of the hen on the day of the first egg laid, in days), egg production (EP, in eggs/hen) and broken egg production (BRKEP, in eggs/hen) were recorded daily from first egg to 38 weeks of age. Egg quality traits of pHu- and pHu+ lines were measured in all eggs for 4 consecutive days at 25, 26, 27, 28, 30, 31, and 32 weeks in G13 and 25, 26, 27, 28, 30, 31, 32, 41, and 42 weeks in G14. Egg weight (EW, in g) was measured with a digital egg tester (DET6000, Nabel Co., Ltd, Kyoto, Japan). Egg shell percentage (ESP, in %) was calculated as egg shell weight / egg weight × 100. Body weight of breeders (BREBW, in g) in females and body weight (BROBW, in g) of female and male broiler chickens was determined individually at 6 weeks of age. The pHu of the right pectoralis major muscle (BRpHu) was measured 24-h after slaughter using a portable pH meter (model 506; Crison Instruments SA, Alella, Barcelona, Spain) by direct insertion of the glass electrode into the thickest part of the muscle as described in [5].

Summary statistics of observed traits subjected to the genetic analysis were performed using the GLM procedure of SPSS. Differences between levels of main effects and their interactions were tested for significance (at $P < 0.05$) using Duncan's test for multiple comparisons. Heritability (h^2) and genetic correlations (r_g) for each trait (except for BRKEP) were estimated by VCE6 software [10] using the restricted maximum likelihood (REML) method and a single-trait and bivariate linear animal model, respectively. As the BRKEP is measured as hatchable or broken-cracked, we used the TM software [11] based on a Threshold Model and Gibbs sampling method for estimating the genetic parameters of this non-normally distributed trait. The following model was used for the genetic analyzes of all traits:

$$y_{ijklm} = \mu + H_i + D_j + S_k + W_l + A_m + e_{ijklm};$$

where y_{ijklm} is the observation of trait *i* for individual *m in the pedigree (m = 7439 birds in pHu- line and 7286 birds in pHu+ line]); μ is the population mean of each genetic line;* H_i is the fixed effect of the i_{th} hatch recoded by generation (*i* = 1 to 39 hatches for each line); D*j* is the fixed effect (only for BRKEP trait) of the j_{th} day in lay (calculated as*: laying day-AFE*); S_k is the fixed effect (only for growth and meat quality traits: BROBW, BRpHu) of the j_{th} sex (*j* = 1 for males and 2 for females); W_l is the fixed effect (only for egg quality traits: EW, ESP) of the l_{th} week (*k* = 25 to 42 weeks); A_m is the random animal genetic effect of trait *i* for individual *m*, and e_{ijklm} is the residual term. A logarithmic transformation for AFE (Log(AFE-140)) was practiced to normalize the distribution of the data before running the genetic analysis. Since a high genetic correlation was detected between AFE and EP in our preliminary analyses, Log(AFE-140) was added as a covariate in estimation of genetic parameters for EP.

Results

Effects of pHu selection on the phenotypic values : Descriptive summary statistics on reproduction, egg quality, growth and meat quality traits in pHu- and pHu+ lines are given in Table 1. The main selection criterion, BRpHu, reached 6.20 in the pHu+ line and 5.56 in the pHu- line from its original value (5.88) in the base population after 14 generations of divergent selection. With this selection, correlated responses were observed in reproduction, egg quality and growth characteristics. The mean AFE, EW and ESP for the pHu- and pHu+ lines were 183.5 and 186.5 days, 55.3 and 58.0 g, and 9.56 and 8.99%, respectively ($P < 0.001$), and generation effect was also significant for these traits ($P < 0.001$). The interaction effect was significant for EP ($P = 0.003$), BRKEP ($P = 0.026$), BROBW ($P = 0.002$), and BREBW ($P < 0.001$) characteristics. Selection of low and high pHu diverged BRpHu between lines from G3, and EP, BRKEP and BREBW from G5 and BROBW from G7. From G5, the pHu- line showed increasing EP and decreasing BRKEP compared to the pHu+ line, with an average of 5 more EP and 1 fewer BRKEP per hen in the pHu- line. The pHu+ line had lower BREBW and BROBW than the pHu- line after the G4 and G6 of pHu selection, respectively.

Genetic parameters of reproductive, growth and ultimate pH according to the genetic line : The heritability of the observed traits and their genetic correlations are given in Table 2. Standard errors of the heritability estimates ranged between 0.01 (for BRKEP) to 0.06 (for AFE). Except for BRKEP, heritability estimates were similar in the two lines and were moderate to high for the different traits (from 0.34 to 0.53). The h^2 value of BRKEP for the pHu+ line (0.16) was more than twice as high as that for the pHu- line (0.07).

Genetic correlations significantly different from zero are indicated in bold. As expected, strong negative genetic correlations were found between AFE and EP whatever the line (r_g of -0.75 and -0.84, in the pHu+ line and pHu- line, respectively). In the pHu- line, a moderate but significant positive genetic correlation was evidenced between BRpHu and AFE (0.32). In turn, BRpHu was negatively correlated with EP (-0.20) in this genetic line. By contrast, no significant genetic relationship was evidenced between BRpHu and AFE nor EP in the pHu+ line. Lowering the genetic level for AFE decreased EW (r_g of 0.43 and 0.37 in the pHu+ line and pHu- line, respectively) and increased ESP (r_g of -0.36 and -0.42 in the pHu+ line and pHu- line, respectively). Indeed, EW and ESP were negatively correlated in the two lines (-0.19 and -0.28 in the pHu+ line and pHu- line, respectively). Quite high negative genetic correlations were found between BRKEP and ESP (-0.77 and -0.53 in the pHu+ line and pHu- line, respectively). BRKEP was also moderately negatively correlated with total egg production (-0.33 and -0.20 in the pHu+ line and pHu- line, respectively). In the two lines, we evidenced that BROBW and BREBW were rather highly positively correlated (0.45 and 0.64 in the pHu+ line and pHu- line, respectively). A moderate but positive genetic correlation was found between BREBW and EW (0.29) and between BROBW and BRKEP (0.22) in the pHu+ line, while no significant correlation was found with any of the laying or egg traits in the pHu- line.

Table 1. Summary statistics of observed traits[1]

Traits[2]	Unit	pHu- line		pHu- line		*P*-values		
		Mean	STD	Mean	STD	Line	Generation	Interaction
AFE	days	183.5[b]	14.0	186.5[a]	14.1	<0.001	<0.001	0.284
EP	eggs	60.5[a]	15.86	55.3[b]	14.9	<0.001	<0.001	0.003
BRKEP	eggs	2.13[b]	3.13	3.38[a]	4.50	<0.001	0.001	0.026
EW	g	55.3[b]	6.56	58.0[a]	6.95	<0.001	<0.001	0.339
ESP	%	9.56[a]	0.90	8.99[b]	0.90	<0.001	<0.001	0.227
BROBW	g	2667.7[a]	396.6	2626.5[b]	401.6	<0.001	<0.001	0.002
BREBW	g	803.8[a]	115.3	754.5[b]	109.6	<0.001	<0.001	<0.001
BRpHu	-	5.66[b]	0.20	6.09[a]	0.20	<0.001	<0.001	<0.001

[1] Data represent estimated marginal (EM) means for each given trait in pHu- and pHu+ line. EM means with different letters within the same row indicate significant differences at the 0.05 level between pHu lines. STD: Standard deviation. [2] AFE: Age at first egg, EP: Egg production, BRKEP: Broken egg production, EW: Egg weight, ESP: Egg shell percentage, BROBW: Broiler body weight, BREBW: Breeder body weight, BRpHu: Breast meat ultimate pH.

Table 2. Estimates of heritabilities (in italic on the diagonal) and genetic correlations (r_g) for reproduction, egg quality, growth and meat quality traits in pHu- (below diagonal) and pHu+ (above diagonal) lines.

Traits	BRpHu	AFE[1]	EP	BRKEP	EW	ESP	BROBW	BREBW
BRpHu	*0.56* *0.50*	0.00	-0.04	-0.00	0.11	-0.02	-0.05	0.05
AFE[1]	**0.32**	*0.40* *0.40*	**-0.75**	0.00	**0.43**	**-0.36**	0.04	-0.14
EP	**-0.20**	**-0.84**	*0.45* *0.36*	**-0.33**	-0.15	**0.35**	0.02	0.16
BRKEP	0.18	0.03	**-0.20**	*0.16* *0.07*	-0.10	**-0.77**	**0.22**	0.01
EW	-0.07	**0.37**	**-0.51**	**0.34**	*0.40* *0.44*	**-0.19**	0.16	**0.29**
ESP	-0.17	**-0.42**	**0.36**	**-0.53**	**-0.28**	*0.49* *0.48*	0.19	0.00
BROBW	0.03	0.15	-0.16	0.08	0.28	0.23	*0.38* *0.34*	**0.45**
BREBW	0.04	0.00	0.07	-0.02	0.15	0.07	**0.64**	*0.53* *0.47*

[1] calculated as Logarithm of (age at first egg-140), EP: Egg production, BRKEP: Broken egg production, EW: Egg weight, ESP: Egg shell percentage, BROBW: Broiler body weight, BREBW: Breeder body weight, BRpHu: Breast meat ultimate pH.

Discussion

Selection for low and high pHu diverged the BRpHu of the pHu lines after the G2. This quick response was due to the high heritability of BRpHu in addition to the fact it was the only selection criterion of the pHu lines. With this selection, the signal of the first physiological changes related to reproduction and growth between the pHu lines could be observed in AFE after G2, EP, BRKEP and BREBW after the G4, and in BROBW after the G6.

In previous studies, the h^2 for AFE ranged from 0.27-0.55 [12,13], and ranged from low to high (0.09-0.43) for EP [13,14,15], in agreement with our study results. The pHu- hens started to lay at an earlier age (3 days) and lay more eggs (~5 eggs) compared to pHu+, which was consistent with Erensoy et al. [16,17]. In our study, higher EP in both lines was genetically associated with early sexual maturity, in agreement with Wolc et al. [12,18], which makes it possible to increase EP for

both lines with selection for an earlier AFE. While selection for BRpHu- was genetically associated with an earlier AFE and a higher EP, no genetic link was found between BRpHu and reproductive functions and egg quality in the pHu+ line. Impairment of laying performance in pHu+ hens is likely due to a mismatch between their metabolic needs and their diet Erensoy et al. [16,17], suggesting a lack of direct genetic linkage between BRpHu and reproductive traits in pHu+ hens.

The h^2 estimates for broken eggs from previous studies ranged from 0 to 0.79, depending on genotype and age of birds, and genetic model used [20]. The heritability of ESP was reported in the range of 0.33-0.53 in previous studies [21,22] and were consistent with our results. The very high negative genetic correlations between BRKEP and ESP for both genetic lines indicate that it is possible to modify BRKEP by selection for ESP. In our study, the greater fragility (higher BRKEP) of pHu+ eggs than pHu- was probably associated with lower ESP [16,17], egg shell weight and breaking strength [19]. Neither lower nor higher pHu selection was directly related to calcium metabolism due to lack of genetic association between BRpHu and BRKEP or ESP, suggesting the existence of different or indirect mechanisms to explain the difference of shell quality between the two lines. Shell quality is related to the ability of eggshells to withstand external factors without breaking or cracking [23]. While ESP is more associated with bird and its associated calcium metabolism, BRKEP is a function involving both pre- and post-ovulation factors, further confirmed by the lower genetic variance (or high environmental variance) of BRKEP than ESP in our study.

The BROBW in the pHu+ line was positively, albeit low, associated with BRKEP of the breeders, suggesting from a practical perspective that selection for body weight in broilers lines with low metabolic reserves (such as the pHu+ line) will result in an increased number of broken and cracked eggs in their breeders. Both this undesirable genetic linkage of BRKEP and the strong genetic correlation between ESP and BRKEP and the high heritability of ESP may be useful selection criteria for producing less fragile eggs in broiler breeders.

EW was moderate to high heritable (h^2 = 0.40-0.44) in pHu lines, which confirms previous studies, for which estimates of heritability for EW ranged from 0.48 to 0.74 [12,24,25,26]. AFE and EW were positively correlated, and EW was negatively associated with ESP. The AFE seems to likely initiate the cascade of variation in egg quality. Because, an earlier AFE is reported to be genetically associated with lower EW and heavier ESP [12,18], which our results confirm for both genetic lines. However, based on the estimated genetic correlations, only in the pHu- line increased muscle energy reserve seems to induce earlier AFE and associated lower EW and higher EP and decreased BRKEP. Along with these, the selection for an earlier AFE means that the number of eggs below standard weight may increase, which would lead to reduction in the number of eggs selected for hatching [27].

The absence of significant r_g between BRpHu and BROBW and BREBW indicates that selection for body growth does not affect breast meat pHu in both lines, and vice versa. However, the selection for BRpHu phenotypically diverged first BREBW (after the G4) and then BROBW (after the G6) between two genetic lines. This may be due to a nutritional program no more adapted to the pHu+ birds requirements [16]. In the pHu+ line, the positive genetic association of BROBW and BREBW with BRKEP and EW, respectively, indicates that selection for accelerated growth may impair egg quality.

Conclusion

The current study showed that genetic selection for pHu diverged the lines phenotypically not only for meat characteristics but also reproduction and egg quality characteristics. Because of favorable genetic correlations, selection for higher muscle energy reserves in the broilers of the pHu- line led to positive correlated responses on reproductive functions (earlier AFE and higher EP) in their breeders, suggesting a better biological efficiency of this genotype. The degradation of egg-laying performances and body weight in female breeders of the pHu+ line could be due to a mismatch between the metabolic needs of these animals and their diet (in quantity and/or quality).

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OP28 The effects of 25-OH D_3 Supplementation On Foot Pad Dermatitis, Slaughter Weight and Carcass Yield in Broilers Exposed to Higher Temperature During Early and Late Term of Incubation Period

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Abstract

This study is performed to investigate the effects of 25-OH D_3 (25-hydroxycholecalciferol) supplementation on foot pad dermatitis, slaughter weight and carcass yield in broilers exposed to higher temperature during early and late term of incubation period. A total of 720 hatching eggs (71.3 g ± 0.8) was used and classified into three experimental groups for incubation temperature treatments: control eggshell temperature (CET: 37.8°C (1-14 days), 36.8°C (15-21 days)), early-term high eggshell temperature (ET-HET: 37.8˚C (1-3 days and 8-14 days), 38.9˚C (4-7 days), 36.8 ˚C (15-21 days)), late term high eggshell temperature (LT-HET: 37.8°C (1-14 days), 38.2°C (15-21 days)). After completing of hatching process, a total of 432 chicks were randomly sampled, and then placed into 18 experimental pens (3 pens/group, 24 chicks/pen) for two dietary groups as control feeding and 25-OH D_3 supplementation. In the control group, commercial starter and grower feeds were used. In the 25-OH D_3 supplementation group, 2500 IU/kg 25-OH D_3 was supplemented into diet. The mild and moderate of foot pat dermatitis was observed with a higher incidence in the ET-HET and LT-HET than the control, whereas a higher incidence of severe foot pat dermatitis was found in the LT-HET group ($P<0.05$). The supplementation of 25-OH D_3 declined both the incidence and severity of foot pad dermatitis. The slaughter and carcass weight were found to be lower in the LT-HET (2934.8 and 2176.1 g) than ET-HET (3177.3 and 2373.6 g) and control (3129.4 and 2328.9 g), whereas the carcass weight and yield was higher in the 25-OH D_3 supplementation group (2349.4 g and 75.5%) than the control (2236.4 g and 73.3%). As a conclusion, the changes in temperature during anytime of incubation period and the supplementation of 25-OH D_3 during post-hatching period had effects on foot pad dermatitis, slaughter, and carcass weight in broilers.

Keywords: incubation temperature, broiler, 25-hydroxycholecalciferol, welfare, carcass yield

IS[11] Causes and Consequences of Intestinal Inflammation in Broilers

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Abstract

Poor gut health is a major issue in modern broiler production. It is characterized by inflammation, dysbiosis and leakage of the intestinal mucosal barrier. It negatively impacts on performance of the birds. Most of the time the birds don't show any signs of disease, other than increased moisture content of the faeces, which may result in wet litter in severe cases. The present review focuses on the different causal factors involved in the initiation and persistence of the inflammatory changes that underlie this complex syndrome. Particular attention is paid to the role of proinflammatory signals of bacterial origin such as the peptidoglycan and the lipopolysaccharide, and the role of pathogens.

Introduction

Commercial broiler lines have been selected for more than half a century for feed intake and feed conversion [1]. The tremendous increase in feed intake and ensuing daily weight gain was not accompanied by an equivalent increase in the development of the digestive system, hence the entire digestive system in continuously under stress in broilers fed ad lib. The cumulative effect of additional stressors both from the environment and from the feed cause damage to the gut barrier, which inevitably leads to gut leakage and inflammation. In the past, these phenomena were largely kept under control by adding small amounts of antibiotics to the feed. Although the mode of action of these so-called growth promoters is still a matter of debate, the positive effects on daily weight gain and feed conversion are undeniable. This improper use of antibiotics, however, is considered now to contribute to the development of multi-resistant strains in many different bacterial species [2]. As a consequence, the use of antibiotics in animal feed has been banned in the E.U. from January 1, 2006. Similar decisions have been made in numerous countries all over the world. Since then, intestinal heath issues, characterized by gut leakage and wet litter, have become a major concern in broiler production and the number one indication for therapeutic antibiotic use [3, 4]. It has generated a renewed scientific interest in the fundamental mechanisms underlying intestinal health. New experimental models for the study of the pathogenesis and for identification of disease biomarkers are continuously being developed [5]. In this paper a brief overview is given of the current state of knowledge about the different known triggers of intestinal inflammation and how this impacts on the bird's health. Some examples are given of how this can be remediated.

Pathogenesis of Intestinal Mucosal Dysfunction

The entire intestinal tract is lined by a single layer of columnar epithelial cells which are sealed together by tight junctions and covered by a protective mucus layer. These together form the gut barrier, separating the host tissues from the intestinal content. The epithelial cells of the small intestine have the challenging task to selectively take up the nutrients from the intestinal lumen through a receptor-mediated uptake mechanism. The absorptive epithelial cells express receptors for every nutrient that is needed for the host metabolism and can be derived directly or indirectly from the feed. At the same time, these same cells need to make sure that potentially harmful molecules from the intestinal lumen don't penetrate through the gut barrier. All of these processes are highly energy demanding and still, the intestinal epithelial cells don't use glucose as their preferred energy source. Instead the epithelial cells of the small intestine prefer glutamine, while epithelial cells of the lower intestinal tract preferentially consume short chain fatty acids provided by the gut microbiota as fuels for their oxidative phosphorylation. The high oxygen consumption by the epithelial cells limits the amount of oxygen diffusing into the gut lumen. Epithelial cells are exposed to a wide variety of noxious agents and substances. Therefore they are continuously renewed. Epithelial cells in the crypts of Lieberkühn between the villi multiply at a high rate, and gradually move up the villi along the basement membrane as they mature. During this process the cells become more specialized. The cells near the tips of the villi are most efficient for absorption of the nutrients. When reaching the villus tip the cells enter into apoptosis, loose contact with the neighbouring cells and are released into the intestinal lumen. Excessive damage and loss of epithelial cells leads to shortening of villi and elongation of crypts, due to the attempt to compensate for the cell loss by enhanced multiplication. While the epithelial cells covering the villi have a high oxygen consumption and don't use glucose, the

dividing cells in the crypts obtain energy through aerobic glycolysis, a process known as the Warburg effect [6]. Epithelial differentiation especially in the lower intestinal tract requires PPAR-g [7], a nuclear receptor which is activated by butyrate, a short chain fatty acid produced especially by members of the Lachnospiraceae and Ruminococcaceae families of bacteria [8]. Loss of butyrate producers thus will lead to lack of oxygen consumption by the epithelium, which further leads to increased oxygen tension in the intestinal lumen [9]. This in turn is harmful for the butyrate producers, which cannot tolerate oxygen, thereby creating a vicious circle of dysbiosis [10]. Damage to the intestinal epithelial cells can trigger the release of proinflammatory cytokines, leading to a powerful inflammatory response. Damage to the tight junction seals between the epithelial cells will lead to leakage of intestinal content between the cells, reaching the subepithelial tissues, where contact with the innate immune sensors, such as the Toll-like receptors (TLR), will also elicit a powerful inflammatory response. This sensor system is even more elaborate in the chicken than in man [11]. Such phenomena can be exacerbated by environmental conditions such as heat stress.

Triggers of Intestinal Inflammation

Bacteria are omnipresent in the biosphere, but they are particularly numerous in chicken houses, where they accumulate during the production cycle [12]. As birds have tendency to scrape and peck from the litter, large numbers of bacteria are continuously swallowed and enter the digestive system, thus creating a continuous threat to gastrointestinal mucosa. The vast majority of these bacteria will be killed by contact with the acid in the crop and proventriculus, creating a continuous flow of dead bacterial cells through the gastrointestinal tract. The digestive enzymes will break down most of the components of these bacterial cells. Some components, however, cannot be broken down by the host enzymes. These include the peptidoglycans, which make up the cell walls of all bacterial cells, and the lipopolysaccharides (endotoxin), which are present in the outer membrane of gram negative bacteria.

Contact of bacterial peptidoglycan with chicken TLR-2 is a strong trigger of the NFkB pathway, which is the main pathway towards inflammation [13]. TLR-2 is strongly expressed in the lower intestinal tract of chickens [14]. We recently studied the effect of adding a microbial enzyme (muramidase) that degrades peptidoglycan, to the diet of broiler chickens [15]. The enzyme was capable of breaking down bacterial peptidoglycan into its smallest biologically active fragment, namely muramyl dipeptide. We further showed that the muramyl dipeptide could activate NOD-2 receptors in eukaryotic cells. Chronic activation of NOD-2 is known to dampen down inflammation. When the muramidase was added to broiler feed, it was found to reduce the inflammatory CD3+ T lymphocyte population in the duodenal mucosa and to expand the anti-inflammatory intraepithelial lymphocyte population and the goblet cell population.

Lipopolysaccharide from gram-negative bacteria is a well known trigger of gut barrier damage and gut inflammation, and is therefore used in experimental gut challenge models in broilers [16]. When in contact with TLR-4, lipopolysaccharide also activates an inflammatory cascade through the NFkB pathway. The release of a range of proinflammatory cytokines downstream this pathway attracts heterophilic granulocytes. These cells also express a wide range of TLRs, thus initiating a vicious circle of inflammation [17].

The small intestinal epithelial cells have the daunting task to take up the nutrients from the intestinal lumen. In addition, they are continuously exposed to many different stressors. Many of these stressors trigger oxidative stress in the epithelial cells. When the production of oxygen radicals exceeds a certain threshold, this leads to excess inflammasome activation and the cell may die through a process of necrosis, which is associated with the release of large amounts of proinflammatory compounds. For review see [18]. Violent cell death is a strong trigger of inflammation [19]. Even minor triggers of inflammasome activation, however, which do not cause cellular necrosis, induce the release of proinflammatory cytokines and thus lead to inflammation [20]. We recently showed that the mere replacement of inorganic zinc by an organic zinc complex in feed reduced oxidative stress and increased villus length in young broiler chickens [21]. This suggests that even the form in which zinc and possibly also other heavy metal trace elements are taken up by the epithelial cells can either or not trigger oxidative stress.

The most powerful triggers of intestinal inflammation are undoubtedly the intestinal pathogens. Some of these are ubiquitous, and thus almost all poultry raised under commercial conditions will be in contact with these at some point in their life. This is definitely the case for coccidiosis. The protozoa that cause coccidiosis are obligate intracellular parasites belonging to the genus Eimeria. Each species targets a specific region in the gut. During their complicated life cycle these parasites cause necrosis of a considerable number of intestinal epithelial cells [22]. Depending on the infection pressure, i.e. number of sporulated oocysts being ingested, the inflammatory trigger thus can be very severe. In addition, the resulting gut barrier leakage leads to plasma protein leaking into the gut lumen, which is associated with dysbiosis [23]. There is a clear association between dysbiosis and inflammation through different pathways [24]. Dysbiosis is commonly associated with a

loss of butyrate-producing Lachnospiraceae and Ruminococcaceae and an expansion of Enterococcaceae families, resulting in reduced butyrate production. Butyrate is known to suppress intestinal inflammation through the PPAR-g pathway, while at the same time inhibiting the expansion of the *Enterobacteriaceae* [25].

Clostridium perfringens is another ubiquitous pathogenic microorganism that may cause damage to the intestinal mucosa in chickens and trigger gut inflammation. This pathogen can express a multitude of virulence factors. Depending on the environmental conditions and on the host, different strains may express different virulence factors to cause diseases known as necrotic enteritis and necrohaemorrhagic enteritis in broilers [26]. All strains express mucinolytic activity, which allows them to penetrate through the protective mucus layer that covers the intestinal epithelium [27]. The first step in this process is the release by the bacterial cells of a sialidase, which cleaves off the terminal sialic acid from the mucin polymer [28]. Surprisingly, the secretion of this sialidase enhances toxin production by the bacteria, thus contributing to overall virulence. Collagenase allows the bacteria to penetrate further into the tissues [29]. In a number of strains coming from different regions, the NetB toxin together with Alpha toxin and some additional virulence factors then cause tissue necrosis. The actual target cell of the toxins in vivo in the chicken is, however, hitherto not identified. In the strains that cause necrohemorrhagic enteritis in broilers, the toxins involved in the necrosis and hemorrhage are not identified yet [25]. Also for the *Clostridium perfringens* strains causing necrotic enteritis in layer pullets, the causative toxin has not been identified yet [30].

Consequences of Intestinal Inflammation

The vicious circle of inflammation in the gut is characterized by an influx and accumulation of inflammatory T-lymphocytes in the lamina propria of the intestinal mucosa, down-regulation of tight junction expression and a switch of the epithelial cell metabolism from high oxygen consumption to low oxygen consumption and high lactate production [31]. As a consequence, oxygen tension in the intestinal lumen sharply rises, causing a shift in the microbial community towards dysbiosis [32]. This drives depletion of butyrate producing bacterial genera and expansion of *Salmonella* [33]. Reduced expression of tight junction proteins by the epithelial cells leads to leakage of the gut barrier. This leakage is even worse under conditions of heat stress [34]. Plasma proteins are then leaking into the intestinal lumen, leading to increased microbial protein fermentation in the gut lumen, which further contributes to dysbiosis and gut barrier dysfunction [35]. Conversely, as mentioned above, fragments from dead bacteria, but also viable bacteria, can migrate from the intestinal lumen into the host tissues and in the bloodstream, thus possibly being transported to distant organs. This phenomenon is called bacterial translocation [36]. Venous blood from the intestinal tissues drains directly to the liver via the portal vein. In the liver the bacterial cells are taken up by macrophages and may cause damage to the liver tissue. Multifocal liver necrosis / hepatitis is a common finding in the broilers seen at slaughter, not only as a consequence of necrotic enteritis as reported by Lovland and Kaldhusdal [37], but also in the absence of necrotic enteritis. In the latter case, different gut bacteria can be cultured from the lesions [38]. Some bacterial species, such as particular strains of *Enterococcus cecorum*, may withstand the host defense mechanisms in the liver and the blood, to reach distant organs such as joints and lumbar vertebral bone marrow, where they induce long standing purulent lesions, as originally described by De Herdt et al. [39]. The link between the Enterococcal spondylitis/arthritis and the ceal microbiota has been established by analyzing the effects of the metaphylactic treatment which was common practice in broiler production [40].

Discussion

Broiler chickens are a valuable source of affordable and good quality meat. As a consequence, consumption of broiler meat is growing worldwide, and this trend is expected to continue in the coming years [41]. In order to be sustainable and reduce the pressure on the environment, broiler production should be as efficient as possible, which implies the lowest possible feed conversion ratio and the highest possible daily weight gain. Intestinal health is absolutely vital for nutrient digestion and absorption, and thus is a determining factor in the performance of broilers. Selection for high growth rate has created an animal with a tremendous appetite, which puts a lot of pressure on intestinal function. Particularly under suboptimal conditions and in the presence of pathogens, this may lead to disturbances of digestion and absorption, dysbiosis and gut inflammation. The intestinal epithelial cells play a pivotal role not only in terminal digestion and absorption of nutrients, but also in regulating intestinal inflammation and gut barrier integrity. As mentioned above, a myriad of factors may interfere with these critical functions and lead to loss of barrier integrity and leakage through the paracellular pathway across the tight junctions. The ensuing inflammatory reaction is not only very energy consuming, it also causes a switch in the epithelial cell metabolism, leading to reduced absorptive function. The accompanying switch in the microbial ecosystem further deteriorates the digestive capacity. These phenomena initially occur without any apparent signs of disease and thus may pass unnoticed while the negative effects on the performance of the birds can be considerable. Once clinical signs appear they are usually characterized by increased water intake and wet litter [42]. Wet litter has a negative impact on performance and welfare of

the birds [43]. At that point the damage is considerable and hard to reverse, even with intensive treatment, hence the interest in developing early warning systems allowing interventions in the first stages of the process. Initially these efforts focused on early detection of increased moisture content of the litter, but it soon became apparent that these methods were not specific and sensitive enough. Also the existing sensors in the poultry house, for example those measuring water consumption, lacked specificity for the detection of intestinal health issues. Most companies therefore still rely on gross gut health scoring during necropsy by veterinarians. Current scientific efforts focus on biomarkers of gut health that can be measured in fresh fecal samples. For review see [44]. This is the topic of another lecture during this conference.

Conclusion

Poor intestinal health is a major challenge in poultry production, affecting the majority of intensively reared broiler flocks. It has negative effects on the performance and welfare of the birds. It is characterized by inflammation, triggered i.a. by proinflammatory bacterial components. Currently novel tools are under investigation which may help to prevent these gut health issues when incorporated in the feed. Further research is needed in order to optimize the control of intestinal health allowing the birds to perform to their full potential.

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OP[29] How Xylanase Alters Arabinoxylan Utilization in Broilers: Characterization of the *In Vivo* Formation of Arabinoxylo-Oligosaccharides

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Abstract

Our previous research has shown the arabinoxylan (AX) depolymerization by xylanase in ileal samples from broilers, but the released arabinoxylo-oligosaccharides (AXOS) were not characterized in detail. The objective of the presented study was to extract and identify the AXOS released *in vivo* in the different segments of the gastro-intestinal tract (GIT) of broilers and demonstrate the influence of xylanase on AX utilization. The study used samples from digesta collected from the gizzard, ileum, ceca and excreta of broilers, fed wheat-soybean meal diet without (CTL diet) or with xylanase supplementation (ENZ diet). AX extractability from ileal digesta of the ENZ diet (26.9%) was higher compared to those of CTL diet (18.8%; $p<0.05$) and influenced the type and amount of AX entering the ceca. Using a recently developed HILIC-MS^n methodology, formed AXOS were identified, AXOS (with degree of polymerization (DP) 4-10) were present in ileum and excreta samples of birds fed ENZ diet, which aligned with improved AX utilization in the hindgut.

Introduction

Cereals are a major energy source in poultry nutrition; other than starch, grains contain non-starch polysaccharides (NSP) present in the cell walls, for instance, wheat contains 9% NSP. Arabinoxylan (AX) is the most abundant NSP, accounting for 5-7% of the grain dry matter. AX may negatively influence broiler performance due to intestinal viscosity increase and nutrient encapsulation. However, soluble AX can be fermented to short chain fatty acids (SCFAs) by gut microbiota in the ceca and benefit of the host (Svihus, Choct, & Classen, 2013). Endo-xylanase supplementation is a successful strategy to offset the anti-nutritive effects of AX. For instance, xylanase-mediated improvements in nutrient digestibility and broiler performance have been associated with reduced digesta viscosity, decreased nutrient encapsulation by the cereal cell wall matrix and pronounced SCFAs formation.

Endo-xylanases hydrolyze the β-(1-4) linkage between two Xyl residues of the AX backbone, releasing (arabino)xylo-oligosaccharides ((A)XOS) (Biely, Vršanská, Tenkanen, & Kluepfel, 1997). The (A)XOS are known for their prebiotic properties but (A)XOS beneficial impact on hindgut fermentation depends on their fine chemical structure (i.e. degree of polymerization (DP), degree/type of substitution) (Mendis, Martens, & Simsek, 2018). Endo-xylanase supplementation has been shown to influence microbiota ecology and to promote cecal SCFAs formation (Masey-O'Neill et al., 2014). Such observations outline that improvement of hindgut fermentation can be expected from the xylanase-mediated degradation of AX to (A)XOS in vivo. However, the detailed characterization of in vivo released AXOS represents a challenge for analyzing such structures in complex digesta matrices and has yet to be performed.In recently published research it was demonstrated that xylanase supplementation in a wheat-based diet led to the *in vivo* formation of pentose oligomers with degree of polymerization (DP) of 5-26 in the proximal GIT of broilers (Kouzounis, Hageman, Soares, Michiels, & Schols, 2021). With the present research we aim to define the structure of AXOS released by diet supplanted xylanase in the broiler GIT and give a detailed characterization of the *in vivo* formed AXOS, to further demonstrate the contribution of xylanase to enhance hindgut fermentation in broilers.

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Materials and Methods

Samples from broiler GIT : Broiler digesta and excreta samples were obtained from an animal study conducted in Belgium in accordance with the ethical standards and recommendations for protection of animals used for experimental studies. Forty-eight (48) one-day old male broilers were reared in a floor pen and fed a common wheat-soybean meal starter (day 0-10) and grower (day 10-20) diets. On day 20, the birds were allocated to different cages and were assigned to control (CTL) or enzyme (ENZ) diets, following a randomized block design. Each treatment had 6 replicate cages, with 4 birds per cage. A control (CTL) or xylanase supplemented (ENZ) wheat-soybean meal finisher diet (66% wheat, 17% soybean meal) was fed till 28 days. The xylanase used was a commercially available endo-1,4-β-xylanase (EC 3.2.1.8) from *Trichoderma spp.* (Huvepharma NV, Berchem, Belgium), used at 1500 EPU/kg feed. Between days 24 and 28 and twice a day excreta were collected per cage and stored at -20°C. On day 28, birds were euthanized and the gizzard, ileum and ceca contents were collected, pooled per cage, and frozen at -20°C.

Preparation of broiler digesta samples for different analytical methods : A mixture of digesta and water was incubated at 99 °C for 20 min and centrifuged. The supernatant was filtered and the residue was added to water, mixed and centrifuged. The extracts and residue were freeze-dried, and the weight of the water extractable solids (WES) and water un-extractable solids (WUS) was recorded. The different fractions were subsequently subject to additional extraction and incubation steps depending on the analytical procedures to be followed:

a) Solid phase extraction (SPE) - used with WES solutions from the gizzard (G_{WES}), ileum (I_{WES}), ceca (C_{WES}) and excreta (E_{WES}). Dried eluted fractions of water (W) or MeOH (M) were coded as G_{WES}-W, I_{WES}-W, C_{WES}-W, E_{WES}-W or G_{WES}-M, I_{WES}-M, C_{WES}-M and E_{WES}-M, respectively.
b) High Performance Anion Exchange Chromatography (HPAEC) – used for analysis of W and M fractions molecular weight distribution, oligosaccharide profile and sugar composition.
c) Hydrophilic interaction liquid chromatography - tandem mass spectrometry (HILIC-MS^n) – ileum or excreta WES (coded: IP_{WES}, EP_{WES}, respectively) fractions were dissolved, incubated and extracted in SPE. The obtained solids were coded as IP_{WES}-M and EP_{WES}-M. The derived solutions were used for the structural analysis of the AXOS and XOS isomers present.
d) Gas Chromatography (GC) – used to determine the neutral sugars composition from whole digesta and WUS fractions after hydrolysis and derivatization of the released sugars.
e) High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) – used for the analysis of the untreated and treated fractions for free monosaccharide content, monosaccharide composition and oligosaccharide profile.
f) High-performance size-exclusion chromatography with refractive index detection (HPSEC-RI) - used for the determination of the molecular weight (Mw) distribution.
g) Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) – used for the characterization of oligomers present (Kouzounis et al., 2021)).

The proportion of AX (sum of Ara and Xyl) in whole digesta from the gizzard, ileum, ceca and excreta that was recovered in WES (WEAX) was determined according to Eq. (1):

Where $AX_{WE,WU}$ is the measured AX content (% dry matter) in WES or WUS of digesta from the gizzard, ileum, ceca and excreta. AX recovery in WUS (WUAX) was determined in a similar manner.

The proportion of AX (sum of Ara and Xyl) ingested by broilers, that was recovered in WES (WEAX) in the ileum and excreta, was determined, according to Eq. (2)

Where AX_{Rec} is the recovery of total AX in the ileum or excreta previously determined using acid insoluble ash (AIA) as a digestibility marker, as described in our recent publication (Kouzounis et al., 2021). Marker-based AX recovery in WUS (WUAX) was determined in a similar manner.

Statistical analysis

Analysis of variance was performed with ANOVA. The significance of differences between treatments was determined by Fisher's test, with significance set at $p<0.05$.

Results and Discussion

Soluble carbohydrate composition varied along the GIT: The monosaccharide composition and total carbohydrate content of WES was determined (Table 1). WES of the gizzard, ileum, ceca and excreta from both diets were composed of approximately 53%, 44%, 11% and 17% carbohydrates (mol %), respectively. Soluble carbohydrates were mainly originated from wheat and soybean meal, that composed 66% and 17% of the diets, respectively. Glucose (Glc), mainly representing starch, was the main water-extractable carbohydrate in the gizzard (79-81% of the neutral sugars), while non-starch carbohydrates Galactose (Gal), Xylose (Xyl), Arabinose (Ara) and Fructose (Fru) were considerably less abundant (4.0-5.4%). Starch digestion in the small intestine decreased the relative amount of Glc and revealed the building blocks of soluble NSP more clearly. Soybean-derived NSP accounted for the high Gal presence in the ileum (19%).

Ara and Xyl together accounted for approximately 20% and 30% of the water-extractable carbohydrates present in the ileum in CTL and ENZ treatments, respectively. These two constituent monosaccharides mainly represented arabinoxylan (AX). Ceca WES presented high free sugar content. Finally, soluble NSP escaping fermentation were still present in the excreta, and were mainly composed of Glc (37%), Ara+Xyl (32%) and Gal (14%).

Table 1. Monosaccharide composition and total carbohydrate content (% w/w) of WES in the gizzard, ileum, ceca and excreta. The monosaccharide proportion (%) as free sugar is shown in parenthesis.

		Monosaccharide composition WES (mol %)								Total (% w/w)
	Treatment	Ara	Xyl	Glc	Fru	Gal	Man	Fuc	Rha	
Gizzard	CTL[a]	4.0 *(6%)*	4.5 *n.d.*	81.1 *(1%)*	4.4 *(23%)*	5.0 *(2%)*	1.0 *n.d.*	0.1 *n.d.*	0.0 *n.d.*	52.9
	ENZ[a]	5.4 *(3%)*	6.0 *n.d.*	78.7 *(1%)*	3.6 *(21%)*	5.2 *(3%)*	0.9 *n.d.*	0.1 *n.d.*	0.0 *n.d.*	52.5
Ileum	CTL	9.3 *(1%)*	10.5 *n.d.*	41.9 *(17%)*	16.2 *(10%)*	18.7 *(14%)*	2.5 *n.d.*	0.9 *n.d.*	0.0 *n.d.*	43.7
	ENZ	12.9 *(1%)*	16.4 *n.d.*	27.7 *(18%)*	19.6 *(18%)*	19.6 *(23%)*	2.7 *n.d.*	1.1 *n.d.*	0.0 *n.d.*	44.2
Ceca	CTL	2.8 *(95%)*	6.7 *(13%)*	60.9 *(48%)*	8.8 *(116%)*	9.9 *(14%)*	3.5 *(112%)*	1.7 *(1%)*	5.6 *n.d.*	10.1
	ENZ	2.7 *(95%)*	5.5 *(13%)*	64.7 *(48%)*	8.3 *(116%)*	8.4 *(14%)*	4.4 *(112%)*	1.3 *(1%)*	4.7 *n.d.*	13.0
Excreta	CTL	14.0 *(7%)*	17.8 *n.d.*	37.5 *(5%)*	10.2 *(28%)*	14.1 *(11%)*	4.5 *n.d.*	2.0 *n.d.*	0.0 *n.d.*	17.4
	ENZ	13.9 *(7%)*	18.2 *n.d.*	37.4 *(9%)*	9.3 *(51%)*	14.3 *(16%)*	4.7 *n.d.*	2.2 *n.d.*	0.0 *n.d.*	17.1

[a]CTL: Control treatment; ENZ: xylanase supplemented treatment

AX solubilisation by xylanase along the GIT: The influence of xylanase on recovery of AX (Eq. (2)) in WES (WEAX) and WUS (WUAX) of ileum and excreta samples was studied (Figure 1). As insoluble digestibility markers are differently retained in the gizzard than soluble feed components, the relative WEAX and WUAX proportion in the gizzard and ceca was determined too (Eq. (1), Table 2). The Ara/Xyl ratio at the different GIT locations was determined as well (Table 3).

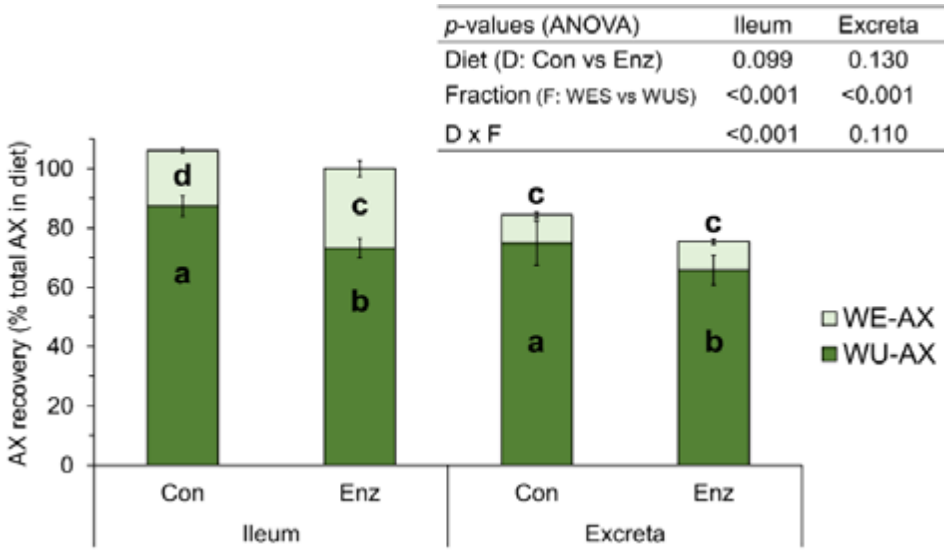

Figure 1. Water-extractable (WEAX) and water-unextractable (WUAX) AX recovery in the ileum and excreta, expressed as percentage (%) of the total AX present in CTL and ENZ diets. Ileum or excreta bard with different notation differ significantly (*p<0.05*). The error bars indicate standard deviation.

WEAX in the ileum represented 18.9% and 26.8% of AX consumed by broilers in CTL and ENZ, respectively (Figure 1). The increase of soluble AX by xylanase in the ileum (*p<0.05*) was followed by a significant decrease in WUAX from 82.3% to 73.2% (*p<0.05*). Comparing the Ara/Xyl values between CTL and ENZ treatments some structural aspects of enzymatically-released AX species are shown, for example, WEAX in the ileum was found to be less substituted for ENZ than for CTL (Ara/Xyl: 0.78 *vs* 0.88; *p<0.05*). In addition, hydrolysis by xylanase resulted in significantly lower WU-AX recovery in excreta, compared to CTL (Figure 1; 65.8% vs 74.9%; $p<0.05$), while ENZ and CTL showed similar WEAX recovery in excreta, with similar Ara/Xyl ratio ($p>0.05$). Overall, the decreased AX recovery from ileum to excreta indicated that xylanase-mediated WU-AX solubilization corresponded to increased WEAX fermentation; the later has been confirmed by a more pronounced acetate and butyrate formation for ENZ compared to CTL for the same ceca samples, reported in previous research (Kouzounis et al., 2021).

Table 2. Total arabinoxylan (AX: sum of Ara and Xyl) recovery (% AX in digesta), and WEAX and WUAX recovery, expressed as percentage (%) of AX present in the gizzard and ceca. Values within GIT location not sharing common notation differ significantly (*p<0.05*).

		Arabinoxylan (AX) recovery (% AX in GIT location)		
		Total	**WE-AX**	**WU-AX**
Gizzard	CTL	91.1	11.7^{b}	88.3^{a}
	ENZ	88.2	13.2^{b}	86.8^{a}
Ceca	CTL	86.6	38.0^{b}	62.0^{a}
	ENZ	117.1	70.2^{a}	29.8^{b}

Overall, the present findings indicated that xylanase increased the proportion of AX being fermented in the ceca (Figure 1, Table 2), which could positively influence SCFAs formation and, consequently, broiler health.

Table 3. Arabinose to xylose ratio (Ara/Xyl) at the different GIT locations (gizzard, ileum, ceca, excreta). (Free Ara and Xyl were excluded from calculation). Values within GIT location not sharing common notation differ significantly (p<0.05).

	Ara/Xyl ratio							
	Gizzard		Ileum		Ceca		Excreta	
	CTL	ENZ	CTL	ENZ	CTL	ENZ	CTL	ENZ
WE-AX	0.85^{a}	0.88^{a}	0.88^{a}	0.78^{bc}	0.13^{c}	0.29^{bc}	0.73^{b}	0.72^{b}
WU-AX	0.65^{b}	0.57^{b}	0.72^{c}	0.81^{ab}	0.35^{b}	0.87^{a}	0.76^{ab}	0.81^{a}

AX and AXOS in the digesta: characterization

a) AX degradation by xylanase starts in the gizzard

Molecular weight (Mw) distribution analysis was performed with purified (A)XOS in the M fractions by HPSEC-RI and oligomers present were profiled by HPAEC-PAD (Figure 2). G_{WES}-M presented different HPSEC profile for ENZ compared to CTL (Figure 2A). Additionally, compounds eluting at similar retention times as AXOS were detected by HPAEC in G_{WES}-M for ENZ only, although the signal was rather low (Figure 2B). Although only a small increase in WEAX was recorded in the gizzard for ENZ compared to CTL (Table 2), it can be concluded that xylanase mainly degraded soluble, polymeric AX in the gizzard to smaller fragments (Mw between 10-100 kDa). These findings show that AX hydrolysis by xylanase began in the gizzard, even when, due to the acidic pH (1.0-4.5) and short feed retention time (30-60 min), it was expected to result in limited activity of supplemented xylanase.

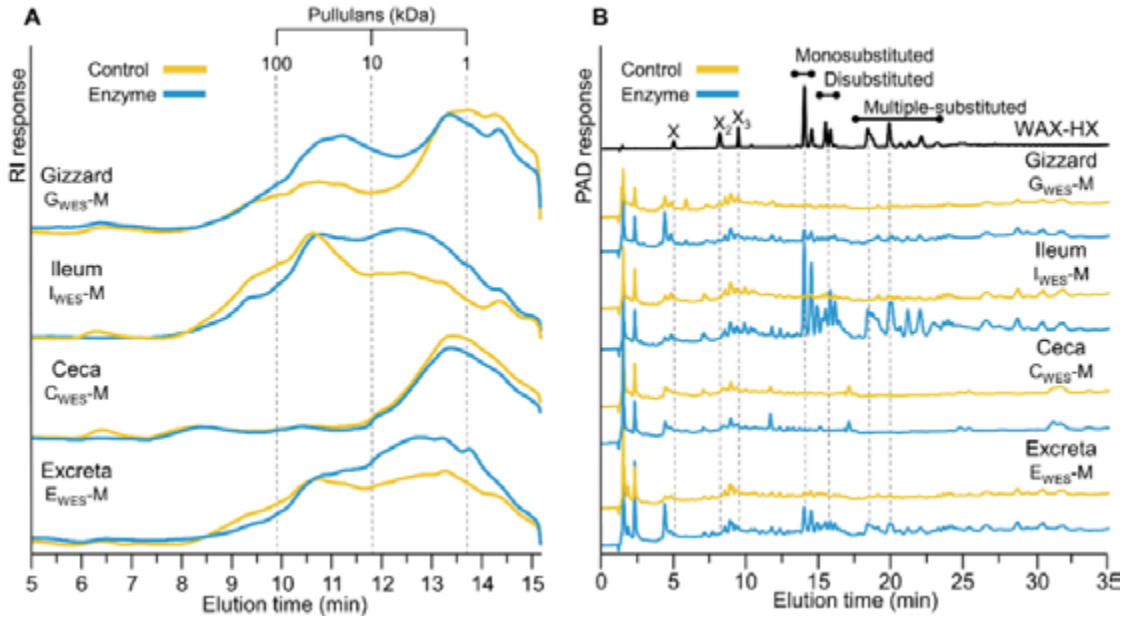

Figure 2. HPSEC-RI (A) and HPAEC-PAD (B) elution patterns of SPE-M fractions of the WES from the gizzard, ileum, ceca and excreta of broilers fed with control and enzyme diets. X: xylose, X2: xylobiose, X3: xylotriose were labelled according to analytical standards; AXOS were eluted in groups of mono-substituted, di-substituted and multi-substituted oligosaccharides (van Gool et al., 2013).2013.

b) *In vivo* formed AXOS: chemical structure

I_{WES}-M for CTL mainly presented molecules between 10-100 kDa Mw, while smaller molecules between 10-1 kDa Mw were more abundant for ENZ (Figure 2A). This shift in size distribution was aligned with AXOS presence in I_{WES}-M for ENZ (Figure 2B). Also relevant, the *in vivo* AXOS profile matched the one obtained during *in vitro* hydrolysis of soluble wheat AX by the same xylanase (Figure 2B; WAX-HX). This supports that AXOS detected in I_{WES}-M for ENZ were formed by the supplemented xylanase. AXOS were also detected in excreta (E_{WES}-M) for ENZ, and presented similar profile to the ileum (Figure 2). The *in vivo* formation of AXOS is in line with the WE-AX depolymerization and with AX solubilization from the water-unextractable cell wall matrix (Figure 1, Figure 4). The enzymatic WUAX degradation *in vivo* offsets nutrient encapsulation by the cell wall matrix, improving nutrient digestibility (Matthiesen et al., 2021). Additionally, AXOS formation in the upper GIT may further explain the positive influence of xylanase supplementation on ceca fermentation processes (Morgan et al., 2019; Singh et al., 2021)cecal short-chain fatty acids (SCFA.

For better understanding of the chemical structure of *in vivo* formed AXOS, the M fractions from ileum and excreta (IP_{WES}-M, EP_{WES}-M) were analyzed by HILIC-MS^n (Figure 3), and the individual AXOS were identified based on a database built-up with retention times and MS^2 and MS^3 mass spectra of known (A)XOS (as described in the recent publication by Kouzounis (Kouzounis et al., 2021)). Ileum and excreta samples presented similar HILIC profiles, with various DP 3-10 (A)XOS isomers being resolved. The observed low substitution degree of AXOS is in agreement with the lower Ara/Xyl ratio observed for WEAX for ENZ treatment compared to CTL (Table 3).

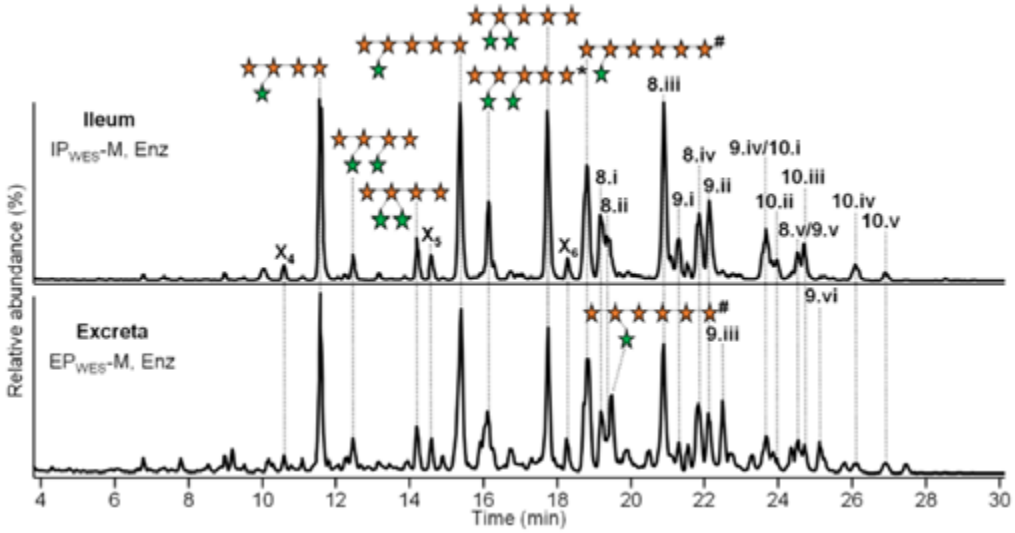

Figure 3. HILIC-MS ion-extracted base peak chromatograms of reduced DP 4-10 (A)XOS present in M fractions of ileum (IP_{WES}-M) and excreta (EP_{WES}-M) samples for ENZ treatment. # general structure; Unidentified DP 8-10 (A)XOS are labelled (i-v) according to their elution order; Arabinosyl (Ara: □) and xylosyl (Xyl: □) units.

c) The fate of *in vivo* formed AXOS in the broiler hindgut. The release of (A)XOS with simple structure by the xylanase in the upper GIT of broilers may further explain the pronounced ceca fermentation previously documented (Kouzounis et al., 2021; Singh et al., 2021)cecal short-chain fatty acids (SCFA. In the current study, soluble compounds with Mw < 10 kDa were present in the ceca (C_{WES}-M), (Figure 2A), however, AXOS were not present (Figure 2B), suggesting that extensive fermentation of both polymeric and oligomeric AX species has occurred in the ceca. Soluble unfermented structures, mainly between 1-10 kDa, were observed in excreta (Figure 2A), suggesting that a WE-AX fraction, including AXOS, passed from the ileum to the hindgut and was excreted unutilized alongside WUAX, without entering the ceca. These findings suggested that fermentable AX-AXOS were not completely utilized in the broiler hindgut.

Conclusion

In this study, xylanase supplementation of broilers diets resulted in AXOS formation *in vivo*. The detection and characterization of released oligosaccharides further delineated the impact of dietary xylanase on hindgut fermentation in broilers. In particular, it is proposed that low-substituted AXOS and XOS, released *in vivo* by the xylanase, were extensively fermented by the ceca microbiota. Further research in terms of AXOS quantification is needed to better understand and optimize AX fermentation. Our work highlights the contribution of dietary xylanase to animal health and provides valuable insight on the utilization of AX and AXOS along the GIT of broilers.

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OP30 Effect of A Precision Biotic on The Growth Performance and Welfare Indicators in Broiler Chickens

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Abstract

Global efforts to reduce antibiotic use in the poultry industry have gathered momentum over the last decade, prompting the development of new technologies to improve animal nutritional health, performance, welfare, and sustainability. Precision biotics (PB) are a new category of nutritional feed ingredients being developed to leverage the recent advances in microbiome science to answer new challenges in the poultry and other protein production industries. The objective of the present study was to evaluate the effect of a PB (Symphiome™, DSM Nutritional Products) on the growth performance and welfare indicators (foot pad dermatitis-FPD) in broiler chickens. A total of 450 one-day-old male Ross 308 broilers were allocated into 30 floor pens located in a commercial broiler house, with 2 treatments, 15 pens per treatment, and 15 broilers per pen. One day after arrival, the chicks were weighed, identified, and distributed into floor pens according to their initial body weight. Environmental temperature was 33°C during the first week and reduced 3°C per week until 21°C, which was maintained until the end of the trial at 35 d. The treatments consisted of feed supplemented or not with 1.43 kg/MT of PB (500 ppm of dried glycans). Feed was based on corn-wheat and soybean meal formulated to meet or exceed the nutritional requirements by the primary breeder (Ross 308) and was supplied was crumble (starter) or pellet (grower and finisher). Phytase was added at 1,000 FYT/Kg. Growth performance was evaluated on d 10, 25, and 35. The feed conversion ratio (FCR) was mortality-adjusted then corrected to a common body weight (cFCR). Mortality was recorded during the entire experimental period. Additionally, FPD was evaluated. The data were analyzed by one-way ANOVA ($P \leq 0.05$), and FPD data was analyzed by Wilcoxson's test by using JMP (16.0). It was observed that the supplementation of PB improved ($P < 0.05$) the feed intake (FI) of the birds during the grower phase and increased the final FI by 5.4% ($P = 0.03$), and tended ($P = 0.10$) to increase body weight gain (BWG) at d 35 by 5.6%. Although not significant, the cFCR at d 35 improved by 3% (~5 points). The number of birds with any FPD lesion score reduced by 15% ($P = 0.05$) in PB supplemented birds. Overall, the results presented herein indicate that the supplementation of PB improved broiler performance, which was found to be at least partially related to reductions in footpad lesions.

Introduction

Global efforts to reduce antibiotic use in the poultry industry have gathered momentum over the last decade, prompting the development of new technologies to improve animal nutritional health, performance, welfare, and sustainability. In parallel, significant advances in molecular and computational biology have also provided a breakthrough in the scientific understanding of the gut microbiome and its metabolic functions across a variety of animal species (1,2). Precision biotics (PB) are a new category of nutritional feed ingredients being developed to leverage the recent advances in microbiome science to answer new challenges in the poultry and other protein production industries (3,4). The mode of action employed by PB is unlike that of probiotics, prebiotics or other conventional gut-health products. Precision biotics are microbiome metabolic modulators (MMM) that influence selected metagenomic functions of the gut microbiome, thereby modulating the production of microbial metabolites targeted specifically to promote beneficial outcomes to the animal and the environment (3,4). Unlike existing technologies that modulate the microbiota, the primary action of PB is through the modulation of the abundance of metabolic pathways rather than the modulation of bacterial taxa, i.e., gut microbial profiles (4).

The reasoning behind PB is to be able to target metagenomic functions, based on a desire to provide a more consistent response than would normally be obtained by using conventional gut health products that target microbial taxonomic abundance. It has been shown conclusively, in humans and other hosts, that there is far less variability in microbiome metabolic function across individuals than there is in their underlying taxonomic composition (5). In other words, the aggregated pathways for core

metabolic functions of the microbiome (6) are often strongly conserved across individuals, even when the relative abundance of different microbial species varies significantly.

Precision biotics are carbohydrates with glycosidic linkages and size distributions selected specifically for their ability to modulate microbiome pathways such as short-chain fatty acid production and amino acid metabolism in the gut (7). For example, it has been found that a specifically selected PB product (Glycan M2-1, Midori USA, Inc., Cambridge, MA, USA, DSM Nutritional Products, Kaiseraugst, Switzerland) modulated the abundance of genes found in microbial pathways associated with propionate production and amino acid metabolism, including pathways that improve energy efficiency (8,9) and reduced intestinal ammonia production, with an improvement observed in growth performance (5).

Footpad dermatitis is characterized by necrotic lesions on the footpads (10), is an important indicator of welfare in broiler chickens and is known to be directly affected by husbandry conditions and farm management (11), and indirectly affected by nutritional management. Additionally, ammonia emissions arising from animal production are under scrutiny due to their negative impact on air quality, eutrophication, and acidification, affecting human health and the health of many terrestrial and aquatic ecosystems (12). Therefore, we hypothesized that the modulation of microbial short-chain fatty acids (SCFA) and amino acid metabolic pathways by Glycan PB would translate into an in vivo reduction of intestinal ammonia and improved litter quality, thus resulting in better animal welfare (e.g., reductions in footpad dermatitis) and reduced environmental emissions from the litter. The objective of this work was to evaluate the effects of a Glycan-based PB on the performance and footpad lesions of broiler chickens. The PB was selected for its ability to activate the propionic acid (C3) and butyric acid (C4) bio-synthesis pathways, and to modulate amino acid degradation and amine biosynthesis. Trial was conducted with commercial-type pens, with an aim to replicate the effects in a subset of end points.

Materials and Methods

A total of 450 one-day-old male Ross 308 broilers were allocated into 30 floor pens located in one commercial broiler house, with 15 broilers per pen. The total area of each pen was 1 m^2, with 0.87 m^2 of usable surface. After disinfection of the buildings and before the arrival of the chickens, new, clean straw pellets were added to the pens to act as bedding material. The chicks were sourced from a local hatchery (Couvoir Josset, Caro, France). One day after arrival, the chicks were weighed, identified, and distributed into floor pens according to their initial body weight (51.1 ± 0.4 g). Environmental temperature was 33 °C during the first week and reduced 3 °C per week until 21 °C, which was maintained until the end of the trial at 35 d. The lighting program gradually increased from 0 h of darkness at placement, to 6 h at d 6. After d 28, darkness hours were gradually reduced to 1 h of darkness at d 35. The ventilation regime followed the breeder guidelines. Trial used a diet based on corn, wheat, and soybean meal, and used a non-starch polysaccharide-hydrolyzing enzyme (1500 viscometric units of endo-1,3(4)-ß-glucanase and 1100 viscometric units of endo-1,4-ß xylanase). All diets contained phytase (1000 FYT/kg).

Trial had a completely randomized design and tested the effect of Glycan PB supplementation using a control diet, and the control diet plus supplementation with Glycan PB at 500 g/MT.

The study variables included BW and cumulative feed intake (FI) at each time of diet change (d 10 and d 28), as well as at the end of the trial at d 35. The feed conversion ratio (FCR) was calculated as the pen total feed intake divided by the pen total weight gain. FCR was mortality-adjusted by adding back the weight of dead birds to the total pen weight and then correcting to a common weight (cFCR) using a correction coefficient calculated from published growth data for the corresponding bird genetics. Mortality was recorded during the entire experimental period. Intra-pen coefficients of variation in BW and footpad lesion scores were also evaluated.

Results

Table 1 shows the effects of Glycan PB supplementation on broiler performance, BW variability, and footpad health from trial. Significant effects of Glycan PB supplementation on FI were observed during 0–25 d and 0–35 d ($p = 0.04$ and $p = 0.03$, respectively), with an increased FI in birds supplemented with Glycan PB. No significant differences were seen for BW gain or cFCR as a result of dietary Glycan PB supplementation. However, Glycan PB tended to increase BW gain by 112 g/bird ($p = 0.10$) over the entire experimental period, and no significant differences in the CVs of BW were detected in trial.

Table 1. Effects of a precision biotic (Glycan PB) ingredient[1] supplemented at 500 g/MT in feed on the growth performance, BW variability, foot pad lesion scores, and mortality of broiler chickens raised in floor cages within a commercial broiler house.

Independent Variable	Negative Control (NC)	NC + Glycan PB	SEM	Probability
		Feed intake (g/bird)		
0–10 d	456	508	28	0.19
0–25 d	1890[b]	2016[a]	42	0.04
0–35 d	2869[b]	3034[a]	51	0.03
		BW Gain (g/bird)		
0–10 d	212	217	2.9	0.28
0–25 d	1164	1187	17	0.33
0–35 d	1882	1994	46	0.10
		cFCR (g feed/g BW gain)		
0–10 d	2.248	2.437	0.146	0.37
0–25 d	1.659	1.708	0.035	0.33
0–35 d	1.594	1.547	0.040	0.42
		BW CV (%)		
10 d	9.40	9.81	0.50	0.57
25 d	12.1	13.1	0.9	0.43
35 d	13.9	12.6	0.9	0.33
		Footpad 35 d		
Score 0 (%)	14.0	27.3	4.6	0.05
Score 1–4 (%)	86.0	72.7	4.6	0.05
Mortality (%)	1.78	3.13	0.96	—

[a,b] Means with different superscripts differed at $p < 0.05$. Superscripts are only shown when main effect of treatment had a $p < 0.05$. (n = 15 pens/treatment). 1 Produced by the catalytic oligomerization of food sugars into tailored glycans (3,7) (Midori USA, Inc., Cambridge, MA, USA, DSM Nutritional Products, Kaiseraugst, Switzerland).

In trial, a numerical reduction in footpad score in response to Glycan PB was observed ($p = 0.10$; Table 1), which was confirmed with a significant increase in the proportion of birds without any signs of footpad dermatitis from 14 to 27.3% ($p = 0.05$), and a reduction of birds with scores between 1 and 4 from 86 to 72.7% ($p = 0.05$).

Discussion

This study evaluated the effects of a Glycan-based PB selected for its ability to activate the C3 and C4 SCFA bio-synthesis pathways and modulate amino acid degradation and amine biosynthesis (4), on the performance and footpad lesions of broiler chickens.

In trial, there was a trend ($p = 0.10$) towards an increase in BW gain by 112 g in in response to Glycan PB supplementation, and a numeral improvement in cFCR (4 points; $p = 0.42$). Clearly, the greater level of variation under commercial conditions affected our ability to detect differences.

In this trial, which was carried out in a commercial environment and exhibited greater variability, supported the findings of another trial (13) with a trend towards a reduction of footpad lesion scores of 0.6 points, and an increase in the proportion of birds with no signs of footpad lesions from 14.0% to 27.3%. In this case, the footpad lesion scores of the control birds were significantly higher compared to another study (13) suggesting a greater challenge for all the birds in trial that might have contributed to this level of variation and a poor overall performance. Interestingly, under these conditions, the main effect of Glycan PB on performance was by increasing FI by 164 g feed/bird, accompanied by an increasing trend in BW gain by 112 g. It is possible that the severity of the challenges on foot health and locomotion may have created a greater potential for the additive, as opposed to feed efficiency, to improve intake and growth.

Conclusions

Overall, the results presented herein indicate that the supplementation of 500 g/MT of Glycan PB improved broiler performance, which was found to be at least partially related to reductions in footpad lesions. Further studies are required to determine the extent to which welfare effects can reflect the reduced ammonia concentrations in the litter versus changes in the production of SCFAs due to the microbiome metabolic modulator used herein. Microbiome metabolic modulators have the potential to modulate the functionality of the gut microbiome by specifically improving the efficiency of microbial carbon and nitrogen utilization, enhancing the sustainability of broiler production by improving broiler welfare and reducing ammonia output to the environment while improving the productivity of the poultry production system.

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OP31 The Synergy of Phytogenic Feed Additives and Esterified Short and Medium-Chain Fatty Acids to Improve Growth Performance in Antibiotic-Free Broilers

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Abstract

For many years, antibiotics have been used in sub-therapeutic doses in animal production to promote animal growth under industrial conditions. However, the misuse of conventional antibiotics for growth promotion has become inappropriate in environmental and public health contexts. In this respect, the use of additives of botanical origin has become more relevant as an alternative method to the use of AGPs, and technologies to improve both human and animal health through natural solutions are becoming increasingly popular. Phytogenic feed additives (PFAs) contain various substances derived mainly from plant extracts and their bioactive compounds. This diversity and synergy with short and medium fatty acids (MCFAs) result in a vast range of physiological effects that have been shown to exert multiple effects on poultry's microbiota and intestinal metabolism.

Therefore, the program of two PFAs in combination with MCFAs was evaluated in broiler chickens under industrial conditions. The trial was conducted on a commercial integration in Central America. Two broiler houses with 10,710 broilers each were evaluated. One house was used as a control group with BMD 11% at the dose of 1,000 g/MT in feed, and in the other group, the Biostrong® program consisting of Biostrong® Forte from d 0-21 at 750 g/MT and Biostrong® 510 from d 22 until the slaughter at 150 g/MT without any addition of growth promoters. Performance parameters such as feed intake, body weight, and feed conversion ratio were monitored throughout the trial. The productive results obtained during the entire experimental period indicate an improvement in all parameters in the group that used the Biostrong® program. Birds receiving the phytogenic additives in feed were slaughtered on day 40, and the animals in the control group left the production facilities for processing on day 42. Hence, the birds in the test group reached the desired weight two days earlier than the control group. Therefore, improved digestion of feed made more nutrients available to the host rather than the bacteria in the intestinal tract. With the data obtained in this trial, it is suggested that the Biostrong® program has considerable potential to improve the growth performance of broilers under industrial conditions by promising results and presenting itself as an excellent tool for those who look for antibiotic-free production.

IS[12] Control of *Salmonella* and *Campylobacter* in Broiler Meat For Sustainable Production

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Globally, diarrhoeal disease has been estimated as the leading cause of death among all ages, with a disproportionate impact on young children (Troeger et al., 2017). One of the principal causes of this disease has been the foodborne hazards, with approximately 550 million cases of illness each year (WHO, 2015). In this sense, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) reported poultry products as the most frequent causative foods of foodborne illness and outbreaks in the European Union (EU) (EFSA and ECDC, 2015). In fact, poultry products have been considered the source for pathogens such as *Campylobacter*, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica, Staphylococcus aureus,* as well as *Bacillus cereus* (Bremner and Johnston, 1996; Heredia and García, 2018). However, *Campylobacter* and *Salmonella* are the leading cause of zoonotic gastroenteritis infections worldwide and the most common causes of human food poisoning in the EU (Rautelin and Hänninen, 2000; CDC, 2019; EFSA and ECDC, 2022). In this sense, the potential for human exposure to *Campylobacter* and *Salmonella* via the poultry production chain has been increased by the ever-growing consumption of poultry products (Clemente et al., 2014). Thus, the control and prevention of these food hazards can be only feasible with proper strategies that minimize them at the poultry production level (Borda-Molina et al., 2018). This is the key to the larger challenge of this extreme growth, where the poultry production intensification cannot be understood without an increase in food safety (Boqvist et al., 2018).

Campylobacter accounts for an estimated annual 96 million cases per year worldwide. Broiler meat is recognized as the most important food vehicle, with illnesses often being due to consumption of raw or undercooked poultry meat (Nastasijevic et al., 2020). Non-typhoidal *Salmonella* (**NTS**) accounts for 80.3 million foodborne cases per year, with 155,000 deaths (Gong et al., 2022). Indeed, EFSA and ECDC considered *Salmonella* as the most common cause of foodborne outbreaks in the European Union (EFSA and ECDC, 2022). Salmonellosis is linked to the consumption of *Salmonella*-contaminated food products mostly from poultry, pork and egg products are considered the most important food vehicles causing strong evidence outbreaks in the EU (EFSA and ECDC, 2022).

In this context, the European policy recommend the control of *Campylobacter* and *Salmonella* from farm to fork, being the first point of control "farm level". In this context, *Campylobacter* affect humans, but not chickens. In this sense, the bacterium could colonize the poultry gut, especially the ceca, in high levels without symptoms, or loss in the production parameters, leading to possible contamination of the poultry meat during slaughter, and subsequent human infections (Lee and Newell, 2006; Hermans et al., 2012; Wagenaar et al., 2013). On the other hand, *Salmonella* is generally classified based on host specificity and infectious nature into two types: typhoidal *Salmonella* and non Typhoidal *Salmonella* (**NTS**). Typhoidal *Salmonella* serovars produce disease in poultry, meanwhile NTS serovars (S. Enteritidis) produce disease in humans but not in poultry.

The concept of *Campylobacter* control in the EU was included in the zoonosis Directive 2003/99/EC (Annex 1), about the prevention and control of the general conditions on the monitoring of zoonoses and zoonotic agents, antimicrobial resistance and foodborne outbreaks (European Commission, 2017). Nevertheless, there was no specific EU legislation for official controls on *Campylobacter* in EU, until 2018. Since then, the Regulation (EC) No 2017/1495 was adopted into EU, amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcases. This enforcement of the food law includes specific microbiological *Campylobacter* criterion within the process hygiene criterion at slaughterhouse level. Under this new European Regulation, the 60% of the broiler carcases tested at a slaughterhouse after chilling must not exceed the 1,000 CFU/g for *Campylobacter.* In order to achieve this objective, good animal handling practices, rigorous cleaning and disinfection protocols, and quality food must be maximized. In addition, it is necessary to be extremely careful during the processing of the carcasses in the slaughterhouse. For *Salmonella* control in poultry, there has been a European

Salmonella control plan since 2005. In this sense, EU regulations have set targets for the reduction of specific serotypes in target population, selected due to their public health significance. For this, strict measures were developed such as the total absence of *Salmonella* on farms (feces and dust), or slaughter of positive breeders and broilers. In order to achieve this objective, good animal handling practices, rigorous cleaning and disinfection protocols, quality food and vaccination must be maximized. Nevertheless, fifteen years after the establishment of the National *Salmonella* Control Plan, the bacterium continues to be one of the main concerns of the industrialized poultry sector. The latest data published in 2021 revealed a total of 127,840 cases of human campylobacteriosis (EFSA and ECDC, 2022). Despite there are multiple *Campylobacter* species, *Campylobacter jejuni* (84.4%) and *Campylobacter coli* (10.1%) are the most commonly implicated species in human cases (EFSA and ECDC, 2022). Moreover, the latest data also revealed a total of 60,050 cases of human salmonelosis (EFSA and ECDC, 2022). Of the more than 2500 serotypes, the 80% of the human infections were related with five *Salmonella* serovars: *S.* Enteritidis (54.6%), *S.* Typhimurium (11.4%), monophasic *S.* Typhimurium (1,4,[5],12:i:-) (8.8%), *S.* Infantis (2.0%) and *S.* Derby (0.93%) (EFSA and ECDC, 2022).

In this context, the control of these bacteria currently presents challenges such as antimicrobial resistance (AMR). In this sense, AMR is one of the most important threats to public health worldwide. Indeed, the World Health Organisation published that by 2050, if effective interventions against the increase in AMR are not carried out, there could be more than 10 million deaths annually as a result of such resistance (WHO, 2019). The first incidence of antibiotic resistance of *Campylobacter* and *Salmonella* was reported in 1990s, and the early 1960s, respectively; these were resistance to a quinolone for *Campylobacter* and chloramphenicol for *Salmonella* (Jajere, 2019). Since then, the isolation frequency of both bacteria resistant to one or more antibiotics has increased globally, with implications of therapeutic failure in cases of life-threatening disease in human, and veterinary medicine (Threlfall et al., 2003; Münch et al., 2012; O 'Neill, 2014; Jajere, 2019). In this sense, *Campylobacter* and *Salmonella* have been included in the World Health Organization priority list of 12 antibiotic-resistant bacteria (Tacconelli et al., 2018).

In the EU, more than 80% of *Campylobacter* isolated from broilers showed resistance to at least one antimicrobial, and approximately 1.3% showed multidrug resistance (EFSA and ECDC, 2020). High prevalence of resistant *Campylobacter* to ciprofloxacin, nalidixic acid and tetracycline was recovered from poultry meat (EFSA and ECDC, 2020). Meanwhile, for *Salmonella* isolates, more than 60% from broiler showed resistance to at least one antimicrobial standing above of laying hens (23.6%) (EFSA and ECDC, 2020). Moreover, approximately 38.2% of broilers and 6.5% of laying hens showed resistance to at least three antimicrobials (EFSA and ECDC, 2020). High prevalence of resistant *Salmonella* to ciprofloxacin, and nalidixic acid sulfamethoxazole and tetracycline. In addition, antimicrobial resistance was evident in more than 59.5% of the chicken carcass (EFSA and ECDC, 2020). Increased awareness of the health threats related to AMR has resulted in greater social demand for antibiotic-free food production, especially antibiotic-free meat, in recent years (Marshall et al., 2011; Chang et al., 2015; Horigan et al., 2016; Liu et al., 2016; Sharma et al., 2018).

In addition to growing concerns about the influence of poultry farming on public health, there is an increasing demand for transparency in animal welfare. This public awareness regarding animal welfare, antimicrobial resistance and environmental health has led to the adaptation of intensive production to more sustainable farming production methods. From the mid-20th century onwards, the majority of chicken production has been intensive, optimising performance through breed selections, shorter generation times, fewer feed conversion ratios and higher densities (Gilbert *et al.*, 2015; Alders *et al.*, 2018; Albrecht *et al.*, 2019). This intensification responds to the demands of a growing and more affluent population for animal-derived products (Gilbert *et al.*, 2015). However, intensive chicken production, which takes place mainly in high-income countries, contrasts with the extensive production mainly by family-based smallholder farms in low-income countries and the co-existence of extensive backyard production with intensive farming in transition economies (Gilbert *et al.*, 2015). Intensified poultry production is mainly carried out by huge sophisticated national or international companies, which are generally highly integrated, that market millions of poultry carcasses annually (McMillin *et al.*, 2012). Nevertheless, this type of production system may entail health risks. The concentration of a large number of animals and the environmental disturbances (temperature, air recycling) facilitate the acquisition, evolution and transmission of diseases (Gilbert *et al.*, 2015). As a consequence, intensive poultry production requires the application of strict biosecurity measures. Moreover, the adaptation to more sustainable production systems faces some unprecedented challenges, such as exposure to adverse weather conditions, cross-infections with wild animals, exposure to predators and risk of endoparasites and other infections (Elson, 2015; Maes et al., 2021). In order to meet these challenges, a "One Health" approach is needed to ensure food safety, control of zoonoses and the fight against antibiotic resistance. In this sense, "One Health" is an approach conceived for design y implement programmes, policies, laws e research at at which multiple sectors can be found at communicate y collaborate to achieve better public health outcomes.

Current approaches are related to coordinated multidisciplinary strategies that aim to develop new antibiotic alternatives combined with improvements in management practices (FDA, 2013). In this sense, the combination of biosecurity measures, as well as vaccination programmes, along with food additives such as probiotics, prebiotics, symbiotics, organic acids, plant extracts and bacteriophages, could play a fundamental role not only in the control and prevention of pathogens such as *Campylobacter* and *Salmonella*, but also in the production parameters, thus complying with market demands. However, it is important to take into account its impact on gut microbiome homeostasis (Thanki *et al.*, 2021; Clavijo *et al.*, 2022). In this sense, gut microbiota plays a key role in vital metabolic functions, with a great impact on host health and performance by modulating physiological processes such as nutrition, metabolism and immunity (Carrasco et al., 2019; Tang *et al.*, 2019; Chen *et al.*, 2019). This complex and dynamic organ is dominated by bacteria, but also includes fungi, archaea, protozoa and viruses. Its knowledge is of increasing scientific interest, as variations in its communities have broad implications for growth efficiency, health, food safety, animal welfare and ecology (Carrasco et al., 2019). In addition, gut microbiota directly regulate host activities through the brain-gut-enteric microbiota axis.

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OP[32] Factors Influencing *Campylobacter* spp. Colonisation of Broiler Chickens*

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Abstract

This study aim to investigate factors influencing *Campylobacter* spp. colonisation of broiler chickens. Campylobacters were isolated from caeca from 319 flocks of two different breeds (199 Cobb and 120 Hubbard), reared as standard (199), Freedom Food/corn fed (57), free-range (47) or organic (16). The standard category exclusively used Cobb birds slaughtered at 38-41 days. The Freedom Food/corn-fed and free-range Hubbard birds were slaughtered at 49-56 days and the organic flocks at 70 days. Campylobacters were picked at random from direct plates. Both breed of chicken (Hubbard) and age at slaughter were independently associated with increased likelihood of colonisation by *Campylobacter coli* rather than *Campylobacter jejuni,* but breed could not be separated from other aspects of husbandry with the data available. Chickens are frequently colonised by *C. jejuni* and *C. coli* and most human infections originate from poultry. In most developed countries approximately 90% of human infections are caused by *C. jejuni*, but fewer than 10% by *C. coli*. This might be due to *C. coli* being less pathogenic than *C. jejuni* to humans, and/or to chicken meat carrying fewer *C. coli* than *C. jejuni.* More investigations are needed into these aspects before it can be concluded that slaughtering older birds from slower-growing breeds would reduce the risk of human *Campylobacter* disease. Meat from certain breeds of poultry are predominantly colonised by *C. coli* rather than *C. jejuni*. More research is needed to understand the impact this may have on the number and severity of human campylobacter infections.

Keywords: age at slaughter, breed, broilers, *Campylobacter coli*, *Campylobacter jejuni*, free-range, organic.

Introduction

Campylobacter spp. are widely regarded as the most common cause of bacterial gastroenteritis in industrialized countries, including Europe (Ketley 1997; EFSA (European Food Safety Authority) 2011; Marotta et al. 2015; Seliwiorstow et al. 2016; EFSA 2017, 2019). Many cases are not reported, and as many as 9 million people are estimated to suffer from campylobacteriosis annually in the EU (Havelaar et al. 2013). The cost of campylobacteriosis for the member countries of the European Union is between 500 and 5000 million euros per year (EFSA 2011; Robyn et al. 2015). *Campylobacter jejuni* and *C. coli* are the most frequently reported species in human cases of *Campylobacter* infection (WHO (World Health Organisation) 2018), causing approximately 90 and 10% of cases, respectively (Gillespie et al. 2002; Nielsen et al. 2006; EFSA & ECDC 2018, 2018; EFSA 2019). The situation is similar in other developed and developing countries (WHO 2018). The sources of human *Campylobacter* infection vary but a significant proportion comes from poultry where these bacteria colonize

the intestine, producing few, if any adverse symptoms in the birds (Corry and Atabay 2001). (EFSA 2010, 2011; Cody et al. 2019; Corry and Atabay 2001).

For standard rearing, modern poultry breeds are selected to grow rapidly in closed poultry houses in order to reduce costs and

meet market-demand as soon as possible. However, intensive rearing can cause problems, including weak legs due to their rapid weight gain, and foot problems associated with poor litter quality (Bessei 2006; Knowles et al. 2008; Granquist et al. 2019). Also, concern among consumers with respect to welfare has encouraged the use of alternative, more welfare-friendly, rearing systems, such as the RSPCA 'Freedom Food' standard (<rspcaassured.org.uk/farm-animal-welfare/>) which include low stocking density, perches and other environmental enrichment, and access to the outside (free range), or provision of organic feed in addition to outside Access ('organic'). These rearing systems are called 'extensive', in contrast to the more common 'intensive' system used for rearing broilers.

In this study we looked at the species of *Campylobacter* isolated from chicken caeca at slaughter and its relation to breed of flock, rearing regime and age at slaughter.

Materials and Methods

Collection of samples : Flocks (319) were sampled from three UK poultry processing plants (A, B, and C) between December 2003 and October 2008. Flocks were defined as all birds originating from the same house/shed on a farm. The flocks comprised two different breeds: Cobb (199 flocks) and Hubbard (120 flocks). The Cobb flocks were all reared intensively as standard birds. Abattoirs A and C processed only intensively reared Cobb flocks (82 and 69 flocks respectively), while Abattoir B processed 48 Cobb flocks and 120 Hubbard flocks. Of the 120 Hubbard flocks, 16 were reared as organic, 47 were reared as free range, while 57 were reared intensively according to the Freedom Food or Freedom Food (Corn-Fed) specifications. The age of the flocks at slaughter varied from 38 to 41 days for the standard (Cobb) flocks, 49 to 56 days for the free range, corn-fed and Freedom Foods (Hubbard) flocks and 70 days for the organic flocks.

Four flocks were selected at random by the processing plant operatives on each sampling day and at least four pairs of caeca were collected from each flock. All caeca were transported to the laboratory on ice, where they were refrigerated, if necessary, prior to analysis. Care was taken to make sure that the caeca were not frozen, which could have inactivated campylobacters, and analysis was carried out within 24 h.

Detection and isolation of *Campylobacter* : All caeca from all the flocks were examined by plating to determine whether or not the flocks were colonized by *Campylobacter*. One caecum from each pair of caeca was placed in a sterile Petri dish and a swab of caecal content was spread directly onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA). Plates were incubated microaerobically in an atmosphere comprising 5–6% oxygen, 3–7% carbon dioxide and 7% hydrogen in a balance of nitrogen, at 41_5°C for 24–48 h. Colonies that had grown under microaerobic but not aerobic conditions were confirmed as *Campylobacter* spp. by a positive oxidase test and the confirmed *Campylobacter* isolates were stored using cryobeads (Microbank) at -80°C prior to further examination.

Speciation of Campylobacter isolates **:** Stock beads were plated onto CBA and incubated in a microaerobic atmosphere at 37°C for 48 h. A DNA template was prepared by suspending a 10 µl loop of culture in 500 µl dH2O and heating at 100°C for 10 min. PCR was carried out according to a modified version of Wang et al. (2002), involving three primer sets designed to identify simultaneously the *hipO* gene from *C. jejuni*, the *glyA* gene from *C. coli* and *23S rRNA* from *Campylobacter* spp. Each PCR reaction contained 25 µl HotStar Taq Master Mix (Qiagen, Manchester, UK), 4 µl $MgCl_2$ (25 mmol l^{-1}), 4 µl primer mix (from stock mix containing 5 µl *C. jejuni* primers, 10 µl *C. coli* primers, 2 µl *23S rRNA* primers and 43 µl nuclease-free water), 1 µl template DNAand 16 µl nuclease-free water to make a final volume of 50 µl. Amplification was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research) under the conditions specified by Wang et al. (2002), with the following modification: an initial denaturation step was carried out at 95°C for 15 min. The PCR products were analysed by gel electrophoresis through 2% (w/v) agarose, containing 1 µl ml^{-1} ethidium bromide, in 1 x TAE buffer. The DNA bands were visualized by means of an ultra-violet transilluminator (BioDoc-It™ Imaging System, UPV). Five microlitres of Hyperladder™ I (Bioline) was used as a molecular marker.

Isolates were confirmed as *Campylobacter* sp. if a band was present at 650 bp (23S rRNA). An isolate was determined as *C. jejuni* or *C. coli* if a band was present at 323 bp (*hipO*) or 126 bp (*glyA*) respectively.

Analysis of results : As all colonies looked similar, the first (or only) colony picked was regarded as a random sample. Results from the first or only isolate picked were first tested for association between the species of *Campylobacter* isolated and breed and rearing regime by chi-squared tests. For samples from which two isolates had been obtained, the dependence of the species isolated (both colonies *C. coli* vs both colonies *C. jejuni*) on breed and age at slaughter (mean-centred days) was further examined by logistic regression analyses. Additionally, multinomial logistic regression was used to include the isolation of one colony of each species. All regressions were tested for goodness of fit by the chi-square method of Hosmer

and Lemeshow (Hosmer and Lemeshow 1989). Calculations were done with SAS version 9.4.

Results

Speciation of isolates : A higher proportion of standard (Cobb) flocks was sampled than non-standard (Hubbard) in all years except for 2008. Isolates (584) were speciated, 403 of which were *C. jejuni*, 178 *C. coli* and three of which were *Campylobacter* species other than *C. jejuni* or *C. coli*.

Overall, *C. jejuni* was the first isolate identified from 72% of flocks while *C. coli* was the first identified isolate from 28% of flocks.

Species of *Campylobacter* in relation to flock type : *Campylobacter jejuni* was more prevalent in Cobb birds reared as standard than in Hubbard birds reared as either free-range (16 flocks), Freedom Food/corn-fed (57 flocks) or organic (47 flocks). Based on the first isolate speciated, there was a significant association between the breed of the chicken flock and the species of *Campylobacter* colonizing the flock (chi-squared test; P < 0_001). Omitting flocks where only one isolate was identified, both *C. jejuni* and *C. coli* were identified from 21 flocks when a second isolate from 121 standard and 102 Hubbard flocks was examined (Table 5). For these 223 flocks there was a significant association between breed and species of *Campylobacter* colonizing the flock (chi-squared test; P < 0_001). All the Hubbard flocks were markedly older at slaughter than the Cobb flocks, and it was clear that there was a correlation between age at slaughter and breed of chicken. These factors were further investigated by logistic regression analysis on data from abattoir B only. The outcomes modelled were: both colonies *C. jejuni* vs both colonies *C. coli.*

Owing to the evident correlation between breed and age at slaughter, the effect of the latter was confirmed by analysing each breed separately with statistically significant results. Colonization by *C. coli* was favoured by later age at slaughter and by breed being Hubbard.

Discussion

Our study examined *Campylobacter*-colonized chickens at slaughter in order to investigate the factor(s) influencing the species (*C. jejuni*, *C. coli* or a mixture of the two species). These factors included the strain of chicken (Cobb or Hubbard), rearing regime (intensive, extensive and diet) and age at slaughter. Significant associations were found between both the strain of chicken (Hubbard more likely than Cobb birds to be colonized with *C. coli*) and age at slaughter (older birds more likely to be colonized with *C. coli*). Both the breed of chicken and the age at slaughter were independently associated with an increasing likelihood of birds becoming colonized by *C. coli* rather than *C. jejuni*, but breed could not be separated from other aspects of husbandry using the data available.

Our finding that the proportion of *C. coli* to *C. jejuni* colonizing the chicken intestine increases with age, concurs with results from several other studies, but our observation that the breed of chicken also influences the predominating species of *Campylobacter*, is new.

The increasing proportion of *C. coli* colonizing chickens during the rearing period is of interest because *C. coli* causes only about 10% of human *Campylobacter* cases while *C. jejuni* causes 90%. Thus, meat from older birds may be less hazardous when consumed than meat from younger birds. There is some evidence that *C. jejuni* strains carry a greater number of virulence genes (Lapierre et al. 2016). Also the fact that Guillain-Barr_e syndrome, a rare and severe disease in humans, sometimes follows a *C. jejuni*, but not a *C. coli* infection (Jasti et al. 2016), indicates that *C. coli* may be less pathogenic. However, meat from these birds would be more expensive than from younger and faster-growing birds. Alternatively, it might be possible to select breeds which become colonized with *C. coli* at an earlier age, and/or to inoculate the chickens with a known low-pathogenic strain of *C. coli*. This would yield cheaper meat.

More investigations are needed into these aspects before it can be concluded that slaughtering older birds from slower-growing breeds would reduce the risk of human *Campylobacter* disease.

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OP33 *Campylobacter* Occurrence in A Poultry Slaughter Line - Strategies To Mitigate Along The Chain

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Abstract

Campylobacter remains one of the most important zoonotic bacterial agents linked to food. Poultry is considered as the main reservoir of *Campylobacter*. The transmission of *Campylobacter* is mainly attributed to the poor biosecurity level in the primary production and once it enters the poultry premises it spreads horizontally very quick as its concentration in feces from infected poultry is very high and it might reach levels of 10^7 per gram. In this study 111 samples were taken out from February 2019 to December 2020 form a poultry slaughterhouse and analyzed for *Campylobacter* within the Processing Hygiene Criteria as laid down the EC regulation 2073/2005 and a legal evaluation of the determined results was given. *Campylobacter* was found in 31 neck samples ranging from 1100 to 70 000 CFU/G and is under the limit (30 % till 2025) of Processing Hygiene Criteria for *Campylobacter*. *Campylobacter* was found in 99 sampling days. In between shift change of water and heating up of the scalding water showed reduction of *Campylobacter*. Reduction in the primary production is the key factor to spread *Campylobacter* along the chain. Once *Campylobacter* establishes in the farm it spread is difficult to be controlled. Use of different acids, biocines and phages has shown to reduce the level of *Campylobacter* in the primary production. However this study shows that poultry might be contaminated with *Campylobacter* and pose a risk for public health and a legal assessment of the associated overall situation. A joint effort along the chain should be aimed to reduce the levels of *Campylobacter*.

Keywords: *Campylobacter*, poultry, slaughter, processing hygiene criteria, legal aspects

OP34 Systematic Evaluation and Meta-Analysis of *Salmonella* Prevalence, Serotype Diversity and Antibiotic Resistance in Poultry Meat in Turkiye

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Abstract

Poultry is one of the most important reservoirs of *Salmonella* and therefore numerous cases related to poultry meat/products and eggs have been reported. In this study, it was aimed to meta-analyze the prevalence, serotypes and antibiotic resistance profiles of *Salmonella* spp. independently detected in poultry meat and eggs consumed in Turkey between 1996 and 2020. In this context, national (ULAKBİM TR Index) and international (Web of Science, PubMed) electronic databases were searched in English and Turkish, and 1818 articles were evaluated. Among them 41 were found eligible to included in the meta-analysis. The pooled prevalence of *Salmonella* in chicken parts, chicken carcass, chicken offal, and eggs was 24.4% (95% CI: 17.8-32.6), 21.9% (95% CI: 14.0-32.7), %20.1 (95% CI: 10.7-34.6) and 4.8% (95% CI: 1.7-13.3), respectively. During the 25-year period, *Salmonella* Enteritidis was detected as the most common serotype in eggs, chicken parts and chicken carcasses, with prevalences of 22.4% (95% CI: 3.6-69.3), 19.0% (95% CI: 3.3-61.6) and 5.8% (95% CI:2.2-14.4). However, in recent studies, it has been observed that *S*. Infantis has become the dominant serotype in poultry meat. The highest antibiotic resistance in *Salmonella* spp. were osbserved for tetracycline (73.9%, 95% CI: 51.0-88.5) and ampicillin (31.5%, 95% CI: 20.7-44, 6). As a result of the study, the pooled prevalence of *Salmonella* in chicken meat and eggs was determined throughout Turkey. It is thought that the results of the study will be useful in establishing the future epidemiological surveillance of the presence of *Salmonella* spp. and antibiotic resistance in poultry meat and eggs in Turkey.

Keywords: *Salmonella*, serotype, meta-analysis, prevalence, antibiotic resistance, poultry meat, egg

IS13 Current Knowledge on Growth-Related Breast Meat Abnormalities in Broilers

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Abstract

Artificial selection for fast-growing and high-breast-yield hybrids has considerably marked up the pressure on breast muscle development, leading to the appearance and expansion of growth-related breast meat abnormalities myopathies (i.e. White Striping, Wooden Breast and Spaghetti Meat) affecting the pectoral muscle of heavy and fast-growing birds. Occurrence of these muscular abnormalities negatively impact both visual aspect and quality properties of raw and processed meat, causing relevant economic damages for the poultry industry. In the past few years, several studies have been carried out to investigate the biological and genetic mechanisms involved in their occurrence with special emphasis on White Striping and Wooden Breast conditions. Main features deal with hypoxia, oxidative stress, dysregulation of energy and carbohydrate metabolism, metabolic shift, vascular damage, and muscle development. It is commonly recognized that the occurrence of growth-related abnormalities boosts with increasing growth rate, slaughter age as well breast yield and weight. Within this context, it seems that artificial selection for broilers growth is close to biological limits and further improvements might be restrained by muscle biological potential and related animal welfare concerns. Thus, attempts have been made in the field of animal nutrition to reduce the occurrence of abnormalities through the modulation of both feed formulation (i.e. dietary supplementation of antioxidants, organic minerals, vitamins and aminoacids) or dietary intake through feed restriction. At slaughter plants, otherwise the most efficient solution seems the incorporation of downgraded meat into the formulation of finely and coarsely minced processed products. In this scenario, it has been also recently suggested that particular attention should be given on the modulation of embryonic formation of additional myofibers, instead of relying on post-hatch selection aimed at increasing muscle mass accretion. This review is therefore intended to make a summary of the possible causative mechanisms and forthcoming methods for mitigation of the most important qualitative issues affecting the chicken meat of fast-growing broilers.

Introduction

Nowadays global meat production derives from a very limited number of mammal and bird species as almost 90% is obtained by chickens (35.4%), pigs (32.6%) and cattle (20.4%) (FAO, 2021). Overall, this shows the gradual loss of biodiversity in the human diet which is increasingly based on a few animal and plant species (Lachat et al., 2018; FAO, 2019). Within this context, poultry production and consumption have increased substantially over the last decades and nowadays poultry meat is one of the primary animal protein sources for many people in several countries (Figure 1) (OECD/FAO, 2021). The universal success of chicken meat is found in its affordability, nutritional and sensory properties, ease of preparation as well as the absence of religious restraints (Baldi et al., 2020).

The development in industrialization and specialization of broiler meat production chains that took place starting from the end of World War II allows to obtain huge advancements in both the efficiency and the chicken meat production (Maharjan et al., 2021; NCC, 2022) (Table 1).

In addition, over the last decades, the lifestyle changes have also dramatically modified the way in which the poultry meat is marketed and consumed and therefore food technologies have become part of the poultry industry, and today much of the production is marketed in the form of cut-up and processed products (Baldi et al., 2020). Indeed, due to the shift of consumers propensity to the convenience of ready-to-cook meals, nowadays almost half of the American meat market involves the commercialization of processed products (Table 1). Because of this market change, the genetic background of modern meat-type chickens has been profoundly adapted by increasing meat-yield and the proportion of high-value parts such as breast (Petracci et al., 2015; Tixier-Boichard, 2020) as shown in Table 2. While the large decline in days to market, coupled with the remarkable boost in breast size, has disclosed huge advancements in broiler productivity, it has also coincided with the development and expansion of muscular defects that affect the breast muscles of fast-growing broiler chickens.

In the past decade, a new group of emerging muscular defects termed as White Striping (WS), Wooden Breast (WB) and Spaghetti Meat (SM) have raised the attention of the scientific community due to their noteworthy incidence levels along with the detrimental implications on meat quality and saleability.

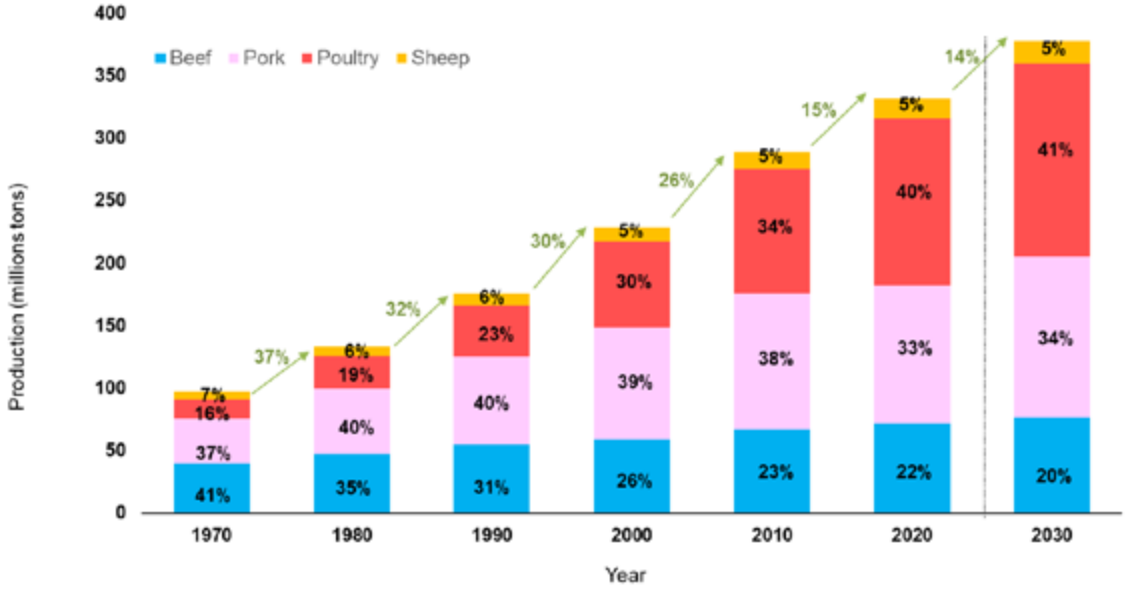


Figure 1. Evolution and projection of global meat production from 1970 to 2030 (Own design, data source: Faostat). Own design, data source: FAO (2022)

Table 1. Progress of broiler performance and evolution of market segments and forms of chicken meat in the US (adapted from NCC, 2022).

Year	Live performances				Market segments		Market forms		
	market age (d)	market weight (kg)	feed to meat gain (kg)	mortality (%)	retail grocery (%)	food-service (%)	whole (%)	cut-up parts (%)	processed (%)
1940	85	1.30	4.0	12	-	-	-	-	-
1950	70	1.40	3.0	8	-	-	-	-	-
1960	63	1.52	2.5	6	-	-	78	19	3
1970	56	1.64	2.25	5	75	25	70	26	4
1980	53	1.78	2.05	5	71	29	50	40	10
1990	48	1.98	2.00	5	59	41	18	56	26
2000	47	2.28	1.95	5	58	42	10	44	46
2010	47	2.59	1.92	4	56	44	12	43	45
2021	47	2.93	1.79	5	55	45	9	40	50

Table 2. Progress in breast weight and yield in chicken strains used for meat production (data referred to male chickens).

Year	Hybrid	Body weight (g)	Age (d)	Breast wt. (g)	Breast yield (%)
1957[1]	ACRBC	1,101	85	133	12.1
2001[1]	Ross 308	2,207	43	349	15.8
2007[2]	Ross 308	2,200	36	410	18.6
2012[2]	Ross 308	2,200	35	464	21.1
2017[2]	Ross 308	2,200	34	484	22.0
2019[2]	Ross 308	2,200	33	516	23.5
2022[2]	Ross 308	2,200	33	530	24.1

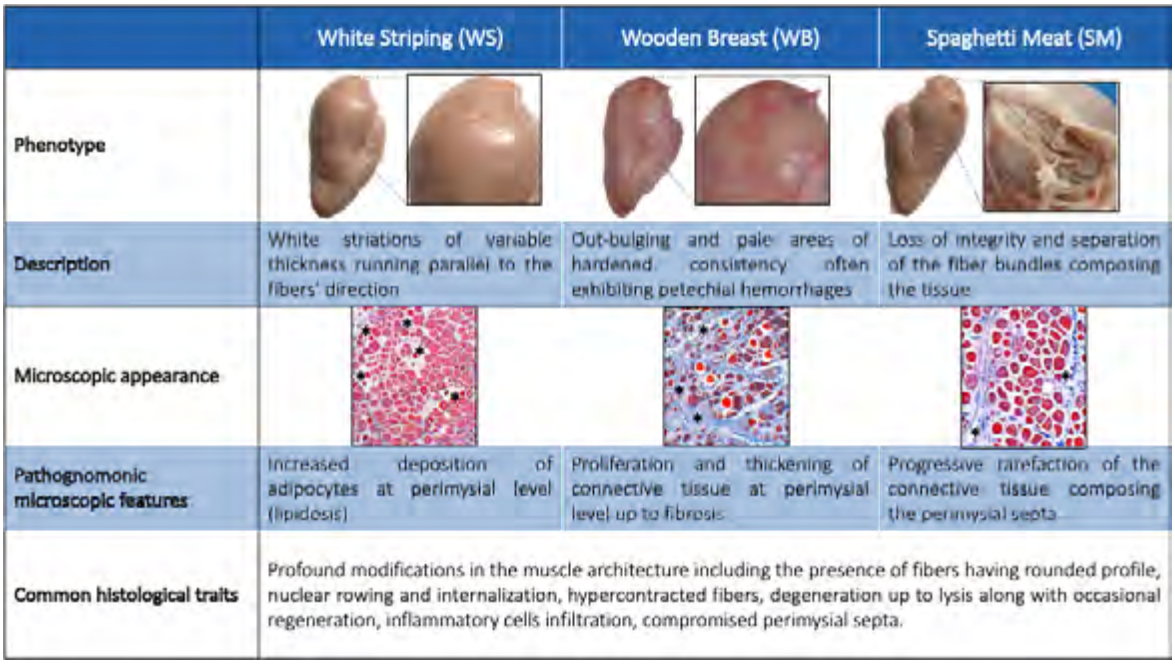
[1]Havenstein et al. (2003); [2]Ross 308 Broiler Performance Objectives

Albeit occurrence levels of affected meat might vary depending on country, animal age and weight at slaughter as well as classification criteria, it is assumed that these muscular defects appear in all countries where fast-growing strains are used

for meat production (Petracci et al., 2019). Phenotypic and microscopic features of the Pectoralis major muscles affected by White Striping, Wooden Breast and Spaghetti Meat conditions are reported in Figure 2. White striping was first noted in 2009 (Kuttapan et al., 2009) and wooden breast myopathy in 2013 (Sihvo et al., 2014), while Spaghetti Meat defect was first recounted in 2015 with the name of "Mushy Breast" by Bilgili (2015). Albeit their distinctive phenotypes, WS, WB and SM conditions entail common histological features, thus suggesting that they might share at least some common causative mechanism that triggers their occurrence (Figure 2).

Nowadays, it is estimated that deboned breast and leg meat (without skin and bone) may reach up to 47% of live weight in birds slaughtered at weigh higher than 3 kg (Aviagen, 2021) (Figure 3). This is contributing to partially counteracting the potential increased proportion of by-products generated at an industrial level due to consumer preference towards trimmed, deboned, ready-to-cook, partially or fully cooked processed products (Baldi et al., 2021).

Figure 2. Phenotype and microscopic traits of broilers' Pectoralis major muscles affected by white striping, wooden breast, and spaghetti meat abnormality (Adapted from Soglia et al., 2021).



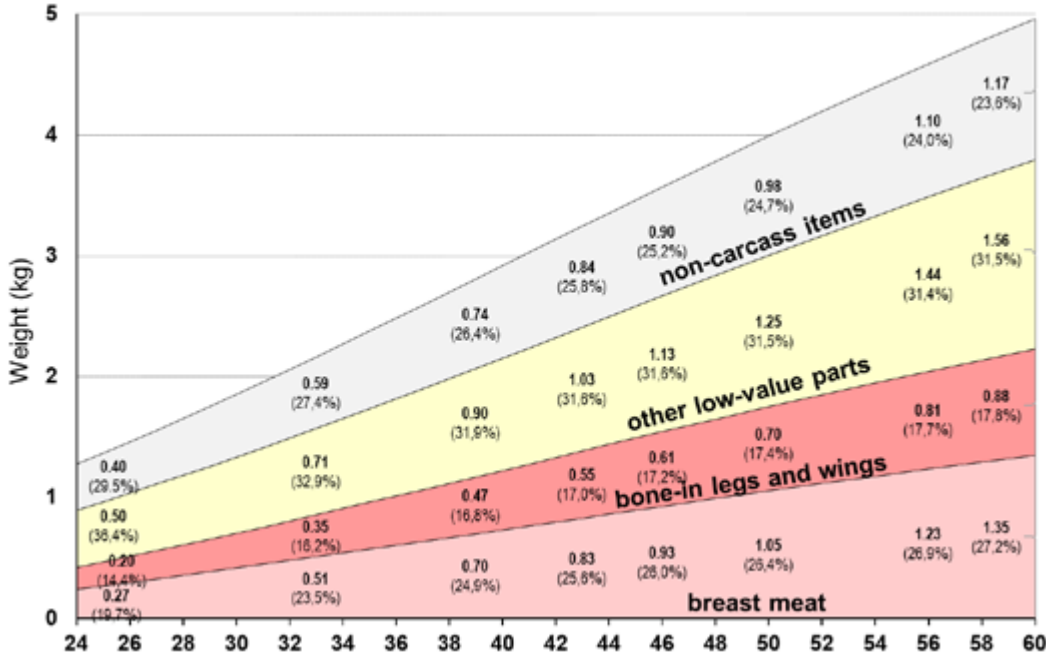


Figure 3. Body composition of male chickens as affected by slaughter weight in Ross 308 hybrid. Own design, data source: Aviagen, 2021.

Origin of growth-related abnormalities

In the past few years, several studies have been carried out with the aim of identifying the causative mechanisms and triggering factors responsible for the onset of the growth-related abnormalities (i.e., WS, WB, SM) and underlying the subsequent network of events leading to their development (Mutryn et al., 2015; Velleman and Clark, 2015; Alnahhas et al., 2016; Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018, 2019; Brothers et al., 2019; Marchesi et al., 2019; Papah and Abasht, 2019; Soglia et al., 2020; Bordini et al., 2021). However, despite the remarkable knowledge gained concerning the microscopic features and the gene expression profile of the affected muscles, the primary cause initiating the process is not clear so far. Indeed, the out- comes of the studies performed at genetic level did not reveal the existence of a major causative gene but rather support a polygenic inheritance of these defects (Pampouille et al., 2018).

Examinations performed at histological level evidenced peculiar features associated with the occurrence of these disorders including an abnormal deposition of adipose tissue (WS), a proliferation and thickening and a progressive rarefaction of the connective tissue (in WB and SM, respectively) at perimysial level (Figure 2). These distinctive histological traits partly account for the phenotypes of the affected muscles which may also profoundly differ. Besides that, the evidence that WS, WB, and SM affected muscles share common microscopic traits seems to support the hypothesis of the existence of a common causative network of events underlying their occurrence (Soglia et al., 2021).

Within this context, a low vascularization of the *Pectoralis major* muscle (due to its hypertrophic growth) is currently considered as the most feasible phenomenon that, leading to hypoxia, likely triggers the development of these disorders (Mutryn et al., 2015; Sihvo et al., 2017; Marchesi et al., 2019; Abasht et al., 2019; Malila et al., 2019; Pampouille et al., 2019; Soglia et al., 2021). Recent findings, further corroborating this hypothesis (Bordini et al., 2021), built a basis for supposing a key role of endoplasmic reticulum stress, responsible for protein folding, in the development of these defects. Indeed, the increase in protein synthesis which is required to support the hypertrophic growth of the pectoral muscle potentially overburdens the capacity of the sarcoplasmic reticulum thus leading to the accumulation of misfolded and/or dysfunctional proteins. This hypothesis is supported by the evidence that alterations in this cellular compartment along with a subsequent activation of the Unfolded Protein Response are among the first ultrastructural and molecular changes associated to an early onset of WB (Papah et al., 2018; Sihvo et al., 2018).

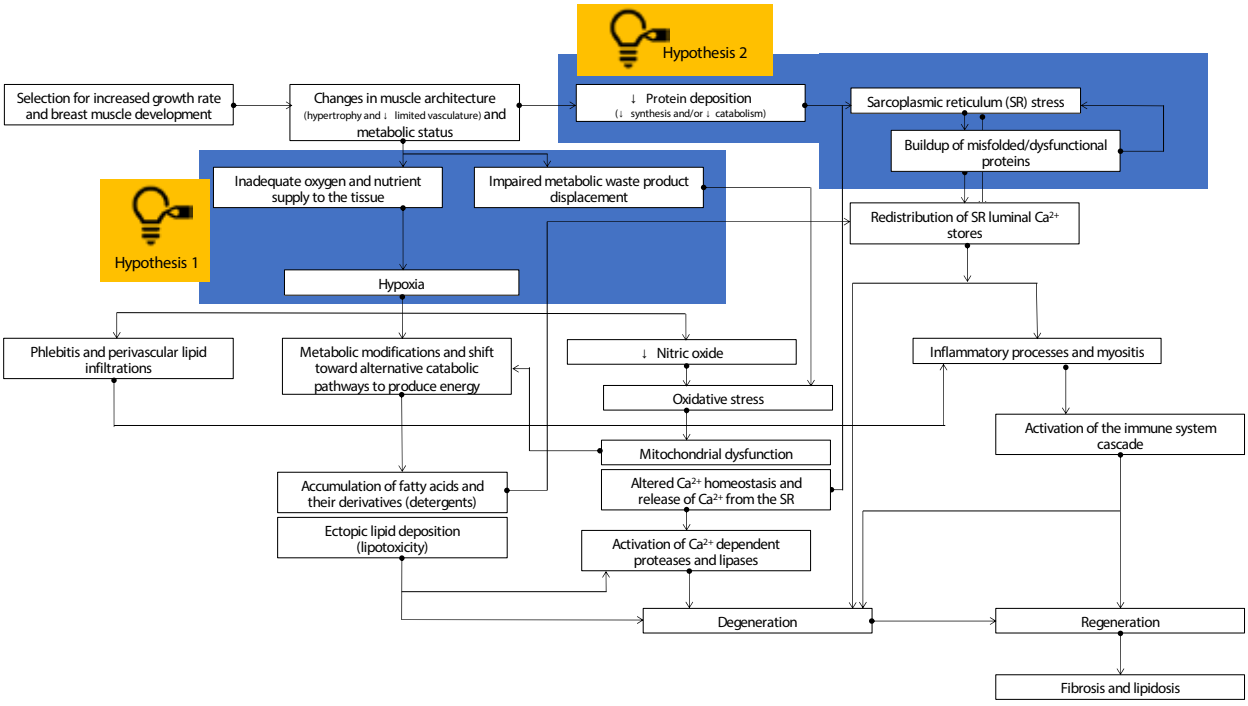


Figure 4. Schematic representation of the possible time series and network of events involved in the onset and triggering the occurrence of the growth-related abnormalities affecting broilers' Pectoralis major muscles (adapted from Soglia et al., 2021).

Then, once that the process has been established, a time-series sequence of events (i.e., phlebitis, oxidative stress, altered calcium homeostasis, etc.) is initiated thus resulting in the activation of complex response mechanisms (i.e., modifications in the energetic metabolism, inflammation, degeneration, and regeneration) (summarized in Figure 4) which ultimately led to the development of the growth-related defects. An extensive description of the network of events likely resulting in the development of WS, WB, and SM abnormalities can be found in the review paper published by Soglia et al. (2021).

Attempts for mitigation

Within this scenario, solutions for avoiding and/or mitigating the occurrence of muscular abnormalities are drawing the attention of the scientific community. The incidence of muscular defects increases with increasing growth rate, slaughter age and weight (Lorenzi et al., 2014; Papah et al., 2017), however Radaelli et al. (2017) reported that the first signs of muscle fiber degeneration associated to muscular abnormalities in fast-growing chickens are visible already at 14 days of age. Thus, attempts in the field of animal nutrition have been made with the purpose of reducing both the severity and the occurrence of WS, WB and SM by the modulation of dietary intake (i.e. feed restriction) or feed formulation (e.g. supplementation of antioxidants, organic minerals, aminoacids, vitamins etc.) (Sirri et al., 2016; Livingston et al., 2018; Zampiga et al., 2018; Bodle et al., 2018; Meloche et al., 2018; Lackner et al., 2022). However, these strategies might not result in any effective mitigation effect because a possible reduction of the incidence of breast abnormalities might be imputable to a decreased slaughter weight and breast size of the animals (Petracci et al., 2019). Thus, incorporating abnormal meat into the formulation of processed products seems the most practical solution, since mincing procedures as well as the addition of functional ingredients might partially conceal the impaired sensory and technological properties of abnormal meat (Carvalho et al., 2021; Santos et al., 2021). Furthermore, since muscular abnormalities mainly affect the superficial section of breast muscles, addressing it for the manufacture of processed products and breast fillet's deep section for fresh retailing could be a strategy to limit the amount of downgraded meat (Baldi et al., 2019). Currently, the interest of scientific research has been also focused on the application of physical (i.e., ultrasounds, pulsed electric field, high pressure processing) and/or chemical (i.e., marinating, enzymes) procedures that might be helpful to improve technological and sensory properties of abnormal meat during processing (Starcevic et al., 2021). A relevant matter for the broiler industry is also the early detection and objective grading of meat affected by muscular abnormalities through reliable and non-destructive methods, which may prevent the need to hire and train on-line personnel. Traffano-Schiffo et al. (2017) proposed radiofrequency spectra as an effective technique to detect WS in chicken carcasses with skin, while hyperspectral imaging (i.e. a novel technique that combines spectroscopy with imaging) was successfully applied to discriminate between normal and WS breast muscles by simultaneously providing information related to chemical and physical characteristics of meat (Jiang et al., 2019). Moreover, NIR spectroscopy lines (Geronimo et al., 2019) and a sideview imaging system (Yoon et al., 2022) have been also used to efficiently detect WB meat in chicken slaughtering lines.

Conclusions

Despite all the efforts made by the scientific community during the past decade, no efficient solutions capable to inhibiting the onset of muscular abnormalities or at least lessening the symptoms and consequences on the quality of the meat have been elucidated. Taking a step back seems unavoidable by now: further pressure exerted on breast muscle development might be restrained by muscle biological potential. In this scenario, albeit solving the issue at its roots appears complex so far, the meat industry will need to re-evaluate selection strategies and opt for more sustainable solutions. In addition, further scientific investigations should be addressed on embryonic formation of additional myofibers, feeding strategies as well as innovative processing solutions aimed at reducing both the economic- and meat quality-related impact of growth-related abnormalities.

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OP[35] Intermittent Dilution of Dietary Digestible Lysine Lowers the Incidence of White Striping by Suppressing the Growth, Lipid Synthesis, and Muscle Damage in Broiler Chickens

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Abstract

Previous studies have shown that intermittent dilution of dietary nutrients suppresses the development of white striping (WS) on the breast muscle of broiler chickens. However, the mechanism by which these interventions reduce the occurrence of WS remains inconclusive. In this study, we adopted intermittent reduction of dietary digestible lysine (dLys) density or metabolizable energy (ME) and amino acid (AA) density using chemical and fatty acid composition of breast fillets, and blood metabolites to understand the mechanism while histopathology and immunohistochemistry of breast muscles were used for confirmation. A total of 300 one-d-old broiler chickens were randomly allocated to 5 experimental, each consisting of 6 replicates with 10 chickens in each replicate. One group served as control group. Remaining groups were fed 85% dLys or 95% ME-AA density diets in grower (GRO 85% dLys or GRO 95%ME-AA) or grower and finisher phases (GRO-FIN 85% dLys or GRO-FIN 95% ME-AA). The experiment lasted for 49 days. Occurrence of white striping was lower in broiler chickens fed 85% dLys diets in comparison with other groups. Crude protein and ether extract in breast meat of 85% dLys groups were greater ($P < 0.001$) and lower ($P = 0.010$), respectively. Serum concentrations of lipid metabolites and enzymes were lower in broiler chickens fed 85% dLys diets than control group ($P < 0.05$). Feeding 85% dLys diets had low degree of myodegeneration and necrosis, inflammation, lipid deposition, infiltration of T-lymphocyte (CD3+) and macrophages (Iba-1+), and low expression of heat-shock protein 70 (HSP70) than other groups ($P < 0.001$). Dilution of dietary dLys to 85% of the required quantities reduces the development of WS in broiler chickens by slowing the growth, lipid synthesis, and muscle damage confirmed by lower extent of histopathological lesions.

Keywords: Broiler chickens; fatty acids; *Pectoralis major*; serum metabolites; myodegeneration; histochemistry

Introduction

Poultry sector has noted a heightened incidence of welfare, meat quality, and economic issues in parallel with improved growth performance, higher edible giblets yield especially breast meat, and shortened production cycle. White striping (WS), one of non-infectious meat quality issues, has emerged as a meat quality issue due to genetic selection and improved feeding of poultry. It is characterized by the appearance of macroscopic fatty white striations/stripes on the surface of *Pectoralis major* muscle that traverse parallel to the direction of myofibers [1]. Several risk factors have been listed that contribute to the development of WS. These include sex, genotype, age, nutrition, body weight, and the growth trajectory of broiler chickens [2]. Due to the faster growth of broiler chickens and accumulation of fatty tissue in the major pectoral muscle, it was thought that the development of WS in broiler chickens may be prevented either by slowing down the growth trajectory or by lowering the accumulation of fatty tissue. Slowdown of growth trajectory in broiler chickens has shown to reduce the occurrence of WS with their own pros and cons. Researchers have made several attempts to slowdown the growth of broiler chickens through quantitative feed restriction [3], and continuous and intermittent restriction of nutrients like metabolizable energy (ME) [4-5], digestible lysine (dLys) [6-8], and amino acids (AA) densities [5, 9]. Continuous restriction of dietary

nutrients greatly affects the growth of broiler chickens while lowering the incidence of WS. Consequently, intermittent restriction of nutrients has emerged as better strategy to reduce or prevent the occurrence of WS in broiler chickens. Previous studies have reported the reduction of WS in broiler chickens in response to intermittent restriction of nutrients (intermittent qualitative feed restriction) without affecting the growth performance of broiler chickens [7,8]. However, no study has reported the mechanism by which intermittent restriction of nutrients lowered the occurrence of WS. Therefore, the present study was conducted to investigate the effect of restriction of dietary dLys, or dietary ME along with AA density during different growth phases on meat quality and composition, serum metabolites, fatty acid composition of breast meat, and pathological responses of WS in broiler chickens. The study was aimed to benefit from the slowdown of growth trajectory of broiler chickens in grower or grower and finisher phases by lowering the dietary nutrient densities (intermittent qualitative feed restriction) followed by provision of required nutrients in order to explain the underlying mechanism by which feed restriction lowers the occurrence of WS.

Materials and Methods

In this study, completely randomized design was followed comprising of 300 one-day-old broiler chickens allotted to five treatments, each consisting of six floor pens as replicates having ten chickens per pen. Nutrient requirements of Ross 308 broiler chickens recommended by Aviagen (Huntsville, AL, USA, 2019) were used as benchmark. Control group was fed starter (days 0–10), grower (days 11–24), finisher (days 25–39), and withdrawal (days 40–49) diets according to the recommendations (Table 1). Remaining groups received diets as follows:

- Two groups were fed diets to meet 85% dietary dLys in grower (GRO 85% dLys) or grower and finisher phases (GRO-FIN 85% dLys), respectively.
- Two groups received diets to meet 95% ME and digestible AA requirements during grower (GRO 95% ME-AA) or grower and finisher phases (GRO-FIN 95% ME-AA), respectively.

The experiment was executed at Poultry Research Center, Aydın Adnan Menderes University, Aydın, Türkiye pursuant to the guidelines of local committee for ethical use of animals in experiments approved vide letter no. 64583101/2021/043 dated March 18, 2021.

Standard management conditions were applied throughout the experiment. Growth performance was recorded in terms of body weight gain, dLys intake, European production efficiency index (EPEF), and European broiler index (EBI). At the end of experiment, all the broiler chickens were slaughtered, breast muscle were scored for the incidence and severity of WS. Chemical composition of breast muscle was analyzed in terms of moisture, crude protein, crude fat, and crude ash. Fatty acid composition of breast meat of broiler chickens was analyzed using gas chromatography. Serum triglycerides (TG), total cholesterol (TC), high- and low-density lipoproteins (HDL and LDL), creatine kinase (CK), alkaline phosphatase (ALP), and aspartate and alanine transaminases were measured to assess the lipid metabolism and muscle damage. Besides these, histopathological and immunohistochemical evaluations of breast muscles were conducted. The data were analyzed using one-way analysis of variance in a computer-based statistical software package (SPSS, version 22.0, Armonk, NY, US).

Results and Discussion

Body weight gain was lower ($P = 0.018$) in broiler chickens fed GRO-FIN 85% dLys diets than those in other groups whereas, broiler chickens fed 85% dLys only in grower phase were lighter ($P = 0.018$) in body weight than control and GRO 95% ME-AA groups (Table 2). Groups fed control and 95% ME-AA diets had greater ($P < 0.001$) dLys intake than those fed GRO 85% dLys diets ($P < 0.001$). The EPEF and EBI were higher ($P = 0.002$) in control and GRO 95% ME-AA than GRO-FIN 85% dLys and GRO-FIN 95% ME-AA.

Table 1. Formulations and chemical composition of diets used for experimental groups (g kg^{-1}, as fed basis).

Ingredients	Starter	Grower			Finisher			Withdrawal
		Control	85% dLys	95% ME-AA	Control	85% dLys	95% ME-AA	
Corn	538.1	593.4	591.2	600.0	608.8	627.3	668.5	649.8
Soybean meal	397.9	339.0	342.5	316.1	313.8	299.0	262.6	280.0
Sunflower oil	22.2	30.2	31.2	15.1	44.8	42.1	14.9	37.8
Sepiolite	-	-	-	30.0	-	-	20.0	-
DCP	17.8	15.4	15.4	16.5	13.2	13.3	13.5	12.8
Limestone	12.0	11.2	11.2	11.5	10.3	10.4	10.5	10.2
Common salt	3.4	3.2	3.3	3.3	3.2	3.2	3.2	3.3
DL-Met	3.0	2.0	2.0	1.8	1.8	1.8	1.6	1.4
L-Lys HCl	2.2	2.3	-	2.4	1.4	-	2.2	1.8
L-Thr	1.4	1.3	1.2	1.3	0.7	0.9	1	0.9
Vit. premix	1	1	1	1	1	1	1	1
Min. premix	1	1	1	1	1	1	1	1
Chemical composition g kg^{-1}, as fed basis)								
Crude protein	231	206	208	198	194	189	184	188
ME,kcal/ kg	3000	3100	3100	2945	3200	3200	3040	3200
Ca	9.8	8.7	8.7	8.8	7.8	7.8	7.8	7.6
Available P	4.9	4.4	4.4	4.4	3.9	3.9	3.9	3.8
dLys	12.8	11.5	9.8	11.0	10.2	8.8	9.7	9.8
dMet	6.0	4.8	4.8	4.5	4.5	4.5	4.1	4.0
dThr	8.6	7.7	7.7	7.4	6.8	6.8	6.4	6.6

Control, GRO and GRO-FIN 95% ME-AA groups had greater breast yield than other treatments ($P < 0.001$). Earlier studies have reported that feeding dietary dLys lower than the recommended levels results in poor body weight gain and growth performance of broiler chickens [6-8]. Poor body weight gain and growth performance is attributable to low dietary dLys that suppresses the feed intake and protein synthesis. It has been explained that broiler chickens, as an adaptive response, tend to reject the diets with depleted essential AA and feeding such diets for longer durations greatly affects the protein synthesis. Low dietary dLys than the required inclusion rate favors the fractional rate of proteolysis thus suppressing the protein accretion [10]. In addition, depletion of any essential AA including dLys in broiler diets exacerbates the initiation stage during the translation process of that inhibits the synthesis of protein in the liver [11]. On the contrary, feed intake is also driven by the ME levels of diets as broiler chickens consume the diets to satisfy their ME requirements [12]. Consequently, in the present study, broiler chickens fed low dLys diets in grower or grower finisher phases exhibited a suppression in the growth performance whereas, feeding 95% ME-AA diets increased the feed intake to compensate the ME depletion of the diets. Occurrence of WS in control, GRO 95% ME-AA, GRO 85% dLys, GRO-FIN 95% ME-AA, and GRO-FIN 85% dLys groups was 76.67% (46/60), 70.00% (42/60), 31.67% (19/60), 73.33% (44/60), and 28.33% (17/60), respectively.

Feeding 85% dLys diets to broiler chickens increased ($P < 0.001$) the CP content and lowered ($P = 0.010$) the EE content of breast meat in comparison with other treatments (Table 3). Most fatty acids were not affected across the treatments. Broiler chickens fed GRO-FIN 85% dLys diets had greater ($P = 0.010$) α-linolenic acid (ALA; C18:3 n-3) compared to other treatments except GRO 85% dLys. Ahsan and Cengiz [8] reported similar findings in broiler chickens fed 85% dLys. Similarly, dilution of dietary ME and CP increased the EE while reducing the CP content in chicken breast meat [13]. It is known that WS occurs due to the myofiber degeneration followed by gradual replacement of degenerated myofiber (protein) by the adipose tissue. Moreover, WS affected breast fillets have greater EE content in addition to lower CP content compared to those without WS [14]. The incidence of WS is lowered in 85% dLys groups than other groups due to slowdown of growth in broiler chickens [8] that was evident in this study as well. Therefore, decreased EE content and increased CP content in 85% dLys groups might be attributed to the fact that these groups had lower incidence of WS than control and 95% ME-AA groups. Most studies dealt with the improvement or evaluation of fatty acid composition of breast meat of broiler chickens in response to different dietary fats and oils. Our study showed no change in the SFA, UFA, MUFA, and PUFA concentrations in the breast fillets across the treatments. This might be due to the fact that all the groups in the present study had breast fillets according to the incidence of WS (both with and without WS). Therefore, most fatty acid contents were not affected except ALA in 85% dLys groups that had lowest incidence of WS.

Table 2. Growth performance and breast yield of broiler chickens

Item	Control	GRO 85% dLys	GRO 95% ME-AA	GRO-FIN 85% dLys	GRO-FIN 95% ME-AA	SEM	*P*-value
Body weight gain, g	3457 [a]	3298 [b]	3424 [a]	3187 [c]	3346 [ab]	20.36	0.018
dLys intake, g	57.0 [a]	52.3 [b]	55.9 [a]	49.0 [c]	55.9 [a]	0.70	<0.001
EPEF	452 [a]	436 [ab]	447 [a]	406 [c]	418 [bc]	4.74	0.002
EBI	446 [a]	430 [ab]	441 [a]	400 [c]	412 [bc]	4.73	0.002
Breast yield, %	76.2 [a]	75.4 [b]	76.3 [a]	75.3 [b]	76.3 [a]	0.12	0.005

[a,b,c] Different superscripts within the same row represent significant difference between the means

Table 3. Nutrient and fatty acid compositions of breast meat of broiler chickens

Nutrients, %	Control	GRO 85% dLys	GRO 95% ME-AA	GRO-FIN 85% dLys	GRO-FIN 95% ME-AA	SEM	*P*-value
Moisture	74.5	74.8	74.7	74.9	74.8	0.07	0.565
Crude Protein	22.2 [b]	22.9 [a]	22.1 [b]	22.9 [a]	22.1 [b]	0.10	<0.001
Ether Extract	1.7 [a]	1.2 [b]	1.7 [a]	1.2 [b]	1.7 [a]	0.05	0.010
Crude Ash	1.2	1.2	1.1	1.2	1.1	0.01	0.269
Fatty Acids, %							
C14:0	0.7	0.9	0.8	0.8	0.7	0.03	0.318
C16:0	22.2	21.5	22.4	22.0	22.9	0.26	0.489
C16:1	1.6	2.1	2.1	2.1	2.3	0.08	0.089
C18:0	11.2	11.6	10.3	10.5	11.2	0.34	0.740
C18:1	27.9	28.1	29.5	29.1	28.8	0.35	0.643
C18:2	34.5	33.5	32.6	33.2	32.2	0.35	0.260
C20:0	0.1 [c]	0.3 [ab]	0.4 [a]	0.2 [bc]	0.1 [c]	0.02	<0.001
C18:3 (n-3)	1.4 [b]	1.6 [ab]	1.5 [b]	1.8 [a]	1.4 [b]	0.04	0.010
C22:0	0.1	0.2	0.1	0.1	0.1	0.02	0.808
C24:0	0.1	0.2	0.1	0.1	0.2	0.02	0.144
SFA[5]	34.4	34.6	34.2	33.7	35.3	0.58	0.947
UFA[6]	65.5	65.4	65.8	66.3	64.7	0.58	0.947
MUFA[7]	29.6	30.2	31.6	31.2	31.0	0.40	0.541
PUFA[8]	35.9	35.2	34.2	35.1	33.7	0.37	0.350

[a,b,c] Different superscripts within the same row represent significant difference between the means

Broiler chickens in control group had greater ($P < 0.001$) serum TG, TC, and HDL levels than GRO and GRO-FIN 85% dLys, and GRO 95% ME-AA groups (Table 4). Serum HDL level was higher ($P < 0.001$) in GRO-FIN 95% ME-AA treatment compared to all other treatments. Feeding diets with 85% dLys in grower or grower and finisher phases of lowered the serum CK ($P < 0.001$), AST ($P = 0.001$), and ALT ($P = 0.010$) levels in broiler chickens in comparison with other treatments. It is believed that lowered dietary dLys density might have reduced the serum lipid metabolites by suppressing the hepatic lipogenesis or lipid biosynthesis which is further supported by the fact that EE content of 85% dLys groups. Consistent with this belief, a recent study showed that broiler chickens fed diets deficient in dLys downregulated the genes encoding hepatic lipid biosynthesis like acetyl-CoA carboxylase and malic enzyme [15]. Serum AST and ALT enzyme levels indicate the status of liver functioning and muscle damage [53], therefore, they might confuse the scenario regarding the source of increase in serum AST and ALT levels that might be attributed to the liver damage or muscle injuries. To avoid this discrepancy, a more specific enzyme CK showing the status of skeletal muscle damage is employed [17]. An increase in serum AST, ALT, and CK concentrations occurred in control and 95% ME-AA groups compared to 85% dLys groups in the present study. This data suggests that the increase in serum concentrations of these enzymes was associated with the muscle damage probably due to the greater occurrence of WS in control and 95% ME-AA groups than 85% dLys groups.

Table 4. Serum metabolites and enzyme concentrations of broiler chickens

Metabolites	Control	GRO 85% dLys	GRO 95% ME-AA	GRO-FIN 85% dLys	GRO-FIN 95% ME-AA	SEM	*P*-value
TG, mg dL^{-1}	56.9 ^{b}	37.8 ^{c}	39.1 ^{c}	45.2 ^{c}	91.5 ^{a}	2.79	<0.001
TC, mg dL^{-1}	180 ^{b}	115 ^{c}	127 ^{c}	124 ^{c}	230 ^{a}	7.53	<0.001
HDL, mg dL^{-1}	64.5 ^{b}	41.1 ^{c}	49.4 ^{bc}	50.2 ^{bc}	82.6 ^{a}	2.72	<0.001
LDL, mg dL^{-1}	25.2 ^{b}	12.6 ^{c}	15.4 ^{c}	15.7 ^{c}	30.6 ^{a}	1.09	<0.001
CK, U L^{-1}	31,997 ^{a}	17,887 ^{b}	33,334 ^{a}	17,966 ^{b}	32,633 ^{a}	1,344	<0.001
ALP, U L^{-1}	1,530	1,282	1,304	1,141	1,506	55.62	0.137
AST, U L^{-1}	264 ^{a}	167 ^{b}	270 ^{a}	173 ^{b}	275 ^{a}	12.01	0.001
ALT, U L^{-1}	6.0 ^{a}	5.2 ^{b}	5.8 ^{b}	5.3 ^{b}	6.0 ^{a}	0.09	0.010

^{a,b,c} Different superscripts within the same row represent significant difference between the means

Broiler chickens fed diets with 85% dLys diets had lower myodegeneration compared to other groups ($P < 0.001$). Infiltration of inflammatory cells, CD3+ T-lymphocytes, and Iba-a+ macrophages was lower in 85% dLys groups than other groups ($P < 0.001$). Lipidosis was lower in broiler chickens fed GRO and GRO-FIN 85% dLys diets in comparison with other dietary treatments ($P < 0.001$). Fibrosis was not detected in any group. Broiler chickens in control and GRO-FIN 95% ME-AA groups had greater ($P < 0.001$) immunoreactivity to HSP70 than other groups. In addition, immunoreactivity to HSP70 was lower ($P < 0.001$) in GRO-FIN 85% dLys group than GRO 85% dLys and GRO 95% ME-AA groups (Table 5).

Table 5. Histopathology and immunohistochemistry of *Pectoralis major* muscle of broiler chickens

Item	Control	GRO 85% dLys	GRO 95% ME-AA	GRO-FIN 85% dLys	GRO-FIN 95% ME-AA[4]	SEM	*P*-value
Myodegeneration	1.5 ^{a}	1.2 ^{bc}	1.4 ^{b}	1.1 ^{c}	1.5 ^{a}	0.028	<0.001
Inflammation	1.3 ^{a}	0.5 ^{c}	1.0 ^{b}	0.4 ^{c}	1.0 ^{b}	0.048	<0.001
Lipid deposition	1.7 ^{b}	1.5 ^{c}	1.9 ^{a}	1.5 ^{c}	1.9 ^{a}	0.037	<0.001
Fibrosis	N/D	N/D	N/D	N/D	N/D	N/D	N/D
CD3+ T-cells	20.9 ^{a}	3.4 ^{c}	13.4 ^{b}	3.1 ^{c}	14.9 ^{b}	1.03	<0.001
Iba-1+	32.1 ^{a}	19.0 ^{c}	24.6 ^{b}	18.1 ^{c}	27.8 ^{ab}	0.88	<0.001
HSP70	71.8 ^{a}	35.7 ^{b}	40.2 ^{b}	20.2 ^{c}	69.9 ^{a}	1.74	<0.001

^{a,b,c} Different superscripts within the same row represent significant difference between the means

Conclusions

Based on the results of this study, it can be concluded that lowering dLys density to 85% of requirement in grower or grower and finisher periods reduces the occurrence of WS in broiler chickens. Reduction in the incidence of WS occurs due to the suppression of growth, carcass and breast yields, and lowered lipid synthesis and muscle damage confirmed by the histopathological and immunohistochemical evaluation of breast muscles. An opposite trend may occur in case of dilution of ME-AA groups due to compensatory feed consumption, thus, failing to reduce the occurrence of WS in broiler chickens.

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OP[36] Restriction of Dietary Digestible Lysine Allowance in Grower Phase Reduces the Occurrence of White Striping in Broiler Chickens

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Abstract

Dietary lysine (Lys) or metabolizable energy (ME) and amino acid (AA) density were reduced during different growth phases to prevent the occurrence of white striping (WS) in broiler chickens. The study consisted of a 49-day rearing experiment involving a total of 390 one-day-old Ross 308 male broiler chickens randomly distributed into 5 experimental groups comprising 30 replicate pens (13 chickens/pen). One group served as control group fed diets according to the nutrient requirements. Two groups were fed 15% low digestible lysine (dLys) in grower (GRO low Lys) or grower and finisher diets (GF low Lys). Other groups received 5% low ME and AA density in grower (GRO low ME AA) or grower and finisher diets (GF low ME AA). Lowering dLys suppressed the growth performance during the respective growth phases. BW gain was lower in GF low Lys group than control group ($P = 0.010$). Broilers in GRO low Lys and GF low Lys groups had lower FI at d 0-49 than those in control group ($P = 0.006$). Birds in control, GRO low Lys, and GRO low ME AA groups showed better FCR ($P < 0.001$). Slaughter weight was lower in GF low Lys group than other groups ($P = 0.003$). Carcass yield was lower in GRO low Lys and GF low Lys groups in comparison with control group ($P = 0.004$). Breast yield was lower in GF low Lys group whereas broilers fed GRO low Lys diets had lower breast yield than those in control group ($P < 0.001$). Meat quality was similar among the treatments except drip loss that was lowest in broilers in control group compared with other groups (P = 0.001). Crude protein increased ($P = 0.045$) whereas crude fat content of breast meat decreased ($P = 0.006$) in broiler chickens fed low dLys diets. Feeding low dLys diets reduced the incidence and severity of WS compared with other groups ($P < 0.001$). It is concluded that reducing the dietary dLys levels during grower phase reduced the development of WS in broiler chickens without affecting the overall growth performance.

Keywords: Broiler chickens; fatty acids; *Pectoralis major*; serum metabolites; myodegeneration; histochemistry

Introduction

Advances in nutrition and genetic selection of chicken have improved the global food security. Simultaneously, these developments have increased the issues of animal welfare, meat quality, and economical losses due to the problems like breast myopathies, modern broiler industry is facing today. White striping (WS) is defined by the macroscopic appearance of fatty white striations between muscle fibers of breast muscle that traverse parallel to the course of muscle fibers [1]. The appearance of WS has adverse effects on the physical and sensory properties of meat [2], thereby reducing consumer acceptance [3], suitability for processing [4], and profitability [1]. Metabolomics of WS confirmed that hypoxia is the main root cause of WS that initiates the development of white striations. Petracci et al. [5] outlined the various predisposing factors including genotype, sex, dietary energy levels, body weight (BW) at slaughter, and growth intensity.

Several attempts have been made to prevent the appearance of WS that have been unable to fully prevent this myopathy in broiler chickens. Since the WS occurs because of fast growth of breast muscles in broiler chickens, the manipulation of growth trajectory is the suitable method to prevent the development of WS. Reduction in nutrient content of diets can slowdown the growth of broiler chickens. Lysine (Lys) is a limiting amino acid (AA) essential for the growth of broilers, protein accretion, and growth of breast muscle [6-8]. Similarly, metabolizable energy (ME) is an essential nutrient for the growth of broiler chickens. Reduction in dietary ME has reduced the incidence of WS in breast meat of broiler chickens [9]. Nevertheless, continuous feeding of diets with lower ME and AA density suppressed the growth performance of broiler chickens [9]. Similarly, continuous reduction of dietary allocation of digestible lysine (dLys) or AA density deteriorates the growth performance, carcass yield, and prevents the development of WS in broiler chickens [10]. In addition, Lys is a basic component of collagen and increased levels of collagen are observed in WS breast meat [11]. Therefore, intermittent decrease

in dietary allocation of dLys or nutrient density might be helpful to prevent the development of WS without disturbing the growth performance. Therefore, the present study was conducted to prevent the incidence of WS in broiler chickens by lowering the dietary dLys or ME and AA density during different growth phases without affecting the growth performance through the manifestation of compensatory growth concept. The objective of this study was to slowdown the growth trajectory of fast-growing broiler chickens at different phases followed by compensatory growth in later phases in order to reduce or prevent the incidence and severity of WS in broiler chickens.

Materials and Methods

In this study, completely randomized design was followed comprising of 390 one-day-old broiler chickens allotted to five treatments, each consisting of six floor pens as replicates having ten chickens per pen. Nutrient requirements of Ross 308 broiler chickens recommended by Aviagen (Huntsville, AL, USA, 2019) were used as benchmark. Control group was fed starter (days 0–10), grower (days 11–24), finisher (days 25–39), and withdrawal (days 40–49) diets according to the recommendations (Table 1). Remaining groups received diets as follows:

- Two groups were fed diets to meet 85% dietary dLys in grower (GRO low Lys) or grower and finisher phases (GF low Lys), respectively.
- Two groups received diets to meet 95% ME and digestible AA requirements during grower (GRO low ME AA) or grower and finisher phases (GF low ME AA), respectively.

All the methods, procedures, and practices involved were in line with the guidelines of local animal care and use committee of the university and approved prior to the commencement of the study vide letter no. 64583101/2018/138 dated December 25, 2018.

Standard management conditions were applied throughout the experiment. Growth performance was recorded for each phase. At the end of experiment, all the broiler chickens were slaughtered, carcass and organ yields were recorded, and breast muscles were scored for the incidence and severity of WS. Chemical composition of breast muscle was analyzed in terms of dry matter, crude protein, crude fat, and crude ash. The data were analyzed using one-way analysis of variance or Kruskal-Wallis test (based on data type) in a computer-based statistical software package (SPSS, version 22.0, Armonk, NY, US).

Results and Discussion

Feeding low dLys diets suppressed the growth of broiler chickens in the corresponding phases that was reflected in the overall growth performance of broiler chickens (Table 2). Earlier studies have reported that feeding dietary dLys lower than the recommended levels result in poor body weight gain and growth performance of broiler chickens [10, 12]. Poor body weight gain and growth performance is attributable to low dietary dLys that suppresses the feed intake and protein synthesis. It has been explained that broiler chickens, as an adaptive response, tend to reject the diets with depleted essential AA and feeding such diets for longer durations greatly affects the protein synthesis. Low dietary dLys than the required inclusion rate favors the fractional rate of proteolysis thus suppressing the protein accretion [6].

Relative liver, heart, and spleen yields were not different among the treatments (Table 3). Slaughter weight was lower in GF low Lys group in comparison with other groups ($P = 0.003$). Carcass yield was lower in GRO low Lys and GF low Lys groups than the control group ($P = 0.004$). Breast yield was lower in GF low Lys group compared with other groups ($P < 0.001$). Thigh yield was lower in GF low ME AA group than GF low Lys groups ($P = 0.041$). Wing yield tended to increase in GRO low Lys and GF low Lys groups in comparison with other groups ($P = 0.057$). Studies reported that *Pectoralis major* muscles are more sensitive to low Lys induced fractional rate of proteolysis than liver [7] and other skeletal muscles like *Anterior latissimus dorsi* (muscle in wings) and *Sartorius* (muscle in thighs) [8]. Also, broiler chickens selected for faster growth and breast muscle development exhibit increased responsiveness of *Pectoralis major* protein turnover to low Lys levels in diet that their slow growing counterpart [13]. Therefore, it seems that breast yield decreased while liver, wing, and thigh yields increased in broiler chickens in GRO low Lys and GF low Lys groups. In nutshell, protein turnover did not help protein deposition in breast muscles at low dietary dLys levels whereas other muscle yields were not affected. Consequently, carcass and breast meat yields in broiler chickens were low or minimum at lower dLys levels than the requirement.

Table 1. Formulations and chemical composition of diets used for experimental groups (g kg^{-1}, as fed basis).

Ingredients	Starter	Grower			Finisher			Withdrawal
		Control	Low Lys	Low ME AA	Control	Low Lys	Low ME AA	
Corn	530.9	557.12	569.31	600.00	611.52	615.06	624.10	600.00
Soybean meal	366.9	360.10	351.80	321.78	302.70	302.06	274.11	260.00
Corn gluten	11.26	–	–	–	–	–	–	35.00
Fish Meal	21.68	–	–	–	–	–	–	6.00
Wheat bran	–	–	–	15.94	–	–	40.00	21.47
Sunflower oil	31.35	45.71	43.36	20.18	51.62	50.48	27.75	46.45
Salt	3.23	3.22	3.21	2.02	3.20	3.20	3.20	3.23
Sod. bicarb	0.18	1.08	1.09	5.43	1.12	1.13	1.11	0.81
DCP	17.36	17.54	17.61	18.57	15.67	15.67	15.65	14.36
Limestone	10.00	8.54	8.55	8.89	7.82	7.82	7.95	7.65
DL-Met	1.84	1.91	1.94	1.93	1.74	1.74	1.57	1.10
L-Lys HCL	2.02	1.76	–	2.12	1.77	–	1.77	1.60
L-Thr	1.31	1.09	1.18	1.19	0.90	0.90	0.83	0.38
Vit. premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Min. premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chemical composition g kg^{-1}, as fed basis)								
Crude protein	231.1	208.4	205.8	197.7	183.8	181.1	178.50	192.30
ME (MJ kg^{-1})	12.56	12.98	12.98	12.37	13.39	13.39	12.72	13.39
Ca	9.90	8.70	8.70	9.00	7.80	7.80	7.80	7.50
Available P	4.80	4.40	4.40	4.50	3.90	3.90	3.90	3.80
dLys	12.80	11.50	9.93	11.00	10.20	8.79	9.70	9.60
dMet	5.10	4.70	4.70	4.60	4.30	4.30	4.07	4.00
dThr	8.60	7.70	7.70	7.40	6.80	6.80	6.50	6.40

Dry matter and crude ash of breast meat were not different among the groups (Table 4). Crude protein was lower in broiler chickens fed low ME AA diets than those fed GRO low Lys diets ($P = 0.045$). Crude fat content of breast meat decreased in broiler chickens fed diets low in dLys (GRO low Lys and GF low Lys) compared to other groups ($P = 0.006$). These findings might be attributed to the pace of muscle growth, and incidence and severity of WS in broiler chickens. It was noted that breast meat showing moderate WS lesions have higher crude fat and lower crude protein percentage than those not having WS lesions and fat and protein content in case of severe WS lesions was even higher and lower than moderate WS breast meat [14]. Under normal circumstances, the muscle growth occurs at a faster pace in modern broiler chickens in response to diets formulated in accordance with their nutrient requirements. It causes the development of localized hypoxia that initiates inflammatory response resulting in muscle fiber degradation [15]. The degraded muscle fibers (protein) are gradually replaced by fat/adipose tissue appearing as WS on the breast muscles running parallel to the muscle fibers. In the present study, the broiler chickens fed diets having low ME and AA density satiated their nutrient requirements through an elevated FI to support the faster growth of muscles. In contrast, birds fed low levels of dietary dLys exhibited lowered FI, slowdown in the growth of muscles, and a low incidence and severity of WS. Consequently, crude fat percentage was higher and crude protein content was lower in broilers fed control and low ME AA diets than those fed low dLys diets.

Table 2. Growth performance and breast yield of broiler chickens

Days	Control	GRO Low Lys	GRO Low ME AA	GF Low Lys	GF Low ME AA	SEM	*P*-value
Body weight gain (g)							
d 0-10	235	210	221	214	225	3.51	0.185
d 11-24	908^{a}	738^{c}	815^{b}	764^{c}	832^{b}	23.29	<0.001
d 25-39	1471^{ab}	1492^{a}	1495^{a}	1315^{c}	1388^{bc}	17.68	<0.001
d 40-49	876	897	915	907	935	14.18	0.781
d 0-24	1143^{a}	947^{d}	1036^{bc}	978^{cd}	1057^{b}	15.94	<0.001
d 0-39	2614^{a}	2439^{b}	2531^{ab}	2292^{c}	2446^{b}	26.52	<0.001
d 0-49	3490^{a}	3327^{ab}	3375^{a}	3193^{b}	3381^{a}	28.24	0.010
Feed intake (g)							
d 0-10	212.32	187.15	201.12	198.51	214.38	3.78	0.134
d 11-24	1243^{a}	1084^{b}	1202^{a}	1106^{b}	1222^{a}	16.95	0.001
d 25-39	2355^{a}	2275^{ab}	2345^{a}	2202^{b}	2382^{a}	21.52	0.042
d 40-49	1687	1705	1682	1678	1778	19.45	0.475
d 0-24	1456^{a}	1271^{b}	1403^{a}	1304^{ab}	1436^{a}	17.89	<0.001
d 0-39	3810^{a}	3546^{b}	3748^{a}	3506^{b}	3818^{a}	36.51	0.003
d 0-49	5497^{a}	5251^{bc}	5430^{ab}	5184^{c}	5596^{a}	43.74	0.006
Feed conversion ratio (g:g)							
d 0-10	0.91	0.89	0.92	0.94	0.97	0.02	0.898
d 11-24	1.37^{b}	1.47^{a}	1.47^{a}	1.45^{a}	1.47^{a}	0.01	<0.001
d 25-39	1.60^{b}	1.53^{c}	1.57^{bc}	1.67^{a}	1.72^{a}	0.01	<0.001
d 40-49	1.95	1.91	1.89	1.88	1.92	0.02	0.797
d 0-24	1.27^{b}	1.34^{a}	1.36^{a}	1.33^{a}	1.36^{a}	0.01	<0.001
d 0-39	1.46^{d}	1.45^{d}	1.48^{c}	1.53^{b}	1.56^{a}	0.01	<0.001
d 0-49	1.58^{b}	1.57^{b}	1.59^{b}	1.64^{a}	1.66^{a}	0.01	<0.001

^{a,b,c,d} Different superscripts within the same row represent significant difference between the means

Table 3. Carcass yield and characteristics of broiler chickens

Item	Control	GRO Low Lys	GRO Low ME AA	GF Low Lys	GF Low ME AA	SEM	*P*-value
Slaughter weight	3566^{a}	3439^{a}	3485^{a}	3299^{b}	3505^{a}	22.71	0.003
Carcass, %	76.38^{a}	75.66^{b}	76.08^{ab}	75.50^{b}	76.44^{a}	0.10	0.004
Breast, %	28.50^{a}	27.30^{b}	28.58^{a}	25.88^{c}	29.12^{a}	0.15	<0.001
Thigh, %	21.04^{ab}	21.32^{ab}	21.18^{ab}	21.62^{a}	20.66^{b}	0.05	0.041
Wing, %	6.78	6.87	6.80	7.02	6.71	0.04	0.057
Liver, %	1.96	2.02	1.96	2.00	1.97	0.02	0.732
Heart, %	0.58	0.60	0.57	0.62	0.59	0.08	0.068
Spleen, %	0.12	0.12	0.12	0.12	0.12	0.02	0.981

^{a,b,c} Different superscripts within the same row represent significant difference between the means

Table 4. Nutrient compositions of breast meat of broiler chickens (%, dry matter basis)

Nutrients	Control	GRO Low Lys	GRO Low ME AA	GF Low Lys	GF Low ME AA	SEM	*P*-value
Dry matter	25.85	25.58	25.63	25.58	25.50	0.08	0.672
Crude protein	87.22^{ab}	87.85^{a}	86.63^{b}	87.50^{ab}	86.67^{b}	0.15	0.045
Crude fat	5.82^{a}	4.66^{b}	5.86^{a}	4.84^{b}	5.81^{a}	0.14	0.006
Crude ash	4.50	4.50	4.48	4.44	4.46	0.01	0.457

^{a,b,c} Different superscripts within the same row represent significant difference between the means

Birds in GRO low Lys and GF low Lys groups had higher frequency and incidence of score 0 and lower frequency and incidence of score 1 in comparison with other dietary treatments ($P < 0.001$) (Table 5). The incidence of severe WS lesions was numerically greater in control, GRO low ME AA and GF low ME AA groups. Feeding low Lys in grower or grower and finisher phases reduced the overall incidence (frequency of total lesions) compared with other groups ($P < 0.001$).

Table 5. Incidence and severity of WS on the breast meat of broiler chickens

Groups	n	White Striping Score			
		0	1	2	Total
Control	78	27^{b} (34.62%)	42^{a} (53.85%)	9 (11.54%)	51^{a} (65.38%)
GRO Low Lys	78	48^{a} (61.54%)	30^{b} (38.46%)	0 (0.00%)	30^{b} (38.46%)
GRO Low ME AA	78	33^{b} (42.31%)	38^{ab} (48.72%)	7 (8.97%)	45^{a} (57.69%)
GF Low Lys	78	47^{a} (60.26%)	31^{b} (39.74%)	0 (0.00%)	31^{b} (39.74%)
GF Low ME AA	78	29^{b} (37.18%)	43^{a} (55.13%)	6 (7.69%)	49^{a} (62.82%)
P-value			<0.001		

^{a,b} Different superscripts within the same row represent significant difference between the means

Broiler chickens that have higher BW, greater BW gain, heavier breast and greater breast yield, faster growth rate, older age, and belonging to male gender are more prone to the development of WS [5]. In protein metabolism, the balance between protein synthesis (anabolism) and protein degradation (catabolism) decides the fate of muscle mass. A balance in the favor of anabolism directs the increase in protein accretion or muscle mass. In modern broiler lines, faster growth rate and muscle accrual is a result of declined protein catabolism that shifts the balance in the favor of anabolism compared with their slow growing counterpart. It has been reported that musculoskeletal defects are attributed to genetic selection for faster growth, effective, and maximal yields [16]. In view of these, it is considered that the muscles expend their full capacity to maintain homeostasis that can be exacerbated in response to any internal or external stress agents. This initiates a chain of muscle fiber degeneration in which fat/adipose tissue steadily replaces the myofiber as in WS. In the present study, broiler chickens fed diets low in dietary dLys levels (GRO low Lys and GF low Lys) slowed down the growth rate by lowering the BW, BW gain, and breast yields, unlike those fed control diets or diets with low ME and AA density (GRO low ME AA and GF low ME AA). Consequently, incidence and severity of WS was lower in broiler chickens fed GRO low Lys and GF low Lys diets in comparison with other groups.

Conclusions

Taken together, it is concluded that reducing the dietary dLys levels during grower phase (GRO low Lys) may reduce the development of WS in broiler chickens by lowering the BW, FI, and fat content of breast meat. This is the first study conducted in commercial broilers and is a torch bearer for further research in this domain. Under commercial settings, rearing period varies among the countries. Therefore, further experiments are recommended to chalk out the optimum dLys restriction levels during an appropriate growth phase to contain the issue of WS in broiler chickens without any adverse effect on growth performance.

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OP37 The Effect of Chia Seed on Fattening Performance and Meat Shelf Life of Broiler Chickens

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Abstract

The aim of this study is to determine the effect of chia seeds added to broiler rations at the level of 5% on live weight, live weight gain, feed consumption, feed conversion ratio, breast meat fatty acid profile and meat shelf life. A total of 112 daily-aged Sasso chicks were used in the experiment. Chicks were divided into 2 groups of 56 animals. Each group is divided into 8 subgroups of 7 broilers. The experimental group ration was prepared to contain 5% chia seeds starting from the 15th day. During the experiment, feed and water were given ad libitum. Before the experiment, a 14-day initial ration was applied to all groups. The trial lasted 49 days. In the study, there was no statistical difference between the groups in terms of body weight, live weight gain, feed consumption and feed conversion rates ($P>0.05$). At the end of the experiment, the breast meat Linolenic acid ratio of the experimental group (2.02%) was significantly higher than the control group (1.07%) ($P<0.05$). Arachidonic acid ratio was found to be significantly lower in the experimental group than in the control group ($P<0.05$). While the TBARS value of breast meats kept at +4°C for seven days increased in the control group, it decreased significantly in the experimental group ($P<0.05$). As a result of this study, it can be said that chia seeds have a positive effect on the shelf life of broiler meat

Keywords: Chia seed, Broiler chick, Meat shelf life, Performance, fatty acid

IS14 Balancing Diet Macro- and Microstructure to Minimize Pelleting Energy Consumption and Optimize Broiler Performance

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Abstract

Feed processing is a complex operation which includes numerous smaller and larger technical operations. The aim of processing is to improve technical and animal performance-related properties of the diet. Grinding is a particularly important processing step, and also adds significant to the cost of feed processing through energy use. For layers the diet is often fed as a mash after mixing the ground ingredients, while pelleting is dominating in poultry meat production, since this improves feed intake and therefore performance. Pelleting is very energy-intensive, accounting for perhaps around 70 % of the total energy consumption in feed processing. The structure of the poultry diets is a major concern, since it affects both technical and nutritional properties. In pelleted diets, structure can be divided into macro- and microstructure. In short, the macrostructure can be defined as the diameter and length of the pellets, plus the amount of and particle distribution of the fraction of pellets that disintegrate between production and feeding. These properties will mainly have an effect on feed intake. The microstructure on the other hand, defines the particle distribution of the particles which the pellet consist of, and is determined by dissolving the pellet in water followed by wet sieving. A sufficiently coarse microstructure is important for gizzard function and thus digestive tract functionality, particularly when diets with a low level of coarse fibre is fed. A delicate interaction exists between macro- and microstructure, complicating optimalisation. However, a coarser grinding, changes to processing conditions and a larger pellet diameter may offer opportunities for considerable reductions in energy consumption and a more healthy and robust digestive tract, while at the same time maintaining broiler performance at the current high levels.

Introduction

Research has with increasing intensity during the last 3 decades emphasized that it is not only ingredient composition and nutrient content that matters for good performance results in poultry. The form and structure of the poultry diet is equally important. It became clear at a very early stage in the emergence of the modern broiler industry that shaping the feed into larger macroparticles in the form of pellets was beneficial. First of all it increased feed intake, but it also reduced feed wastage, while at the same time assuring that the birds were not able to selectively eat and thus that they were eating all the feed components of the more and more optimized modern poultry diet. An additional advantage of pellets was that density increased 25 to 30 percent, and the flow properties increased, making handling and conveying of the feed easier. For some countries with strict hygiene requirements, pelleting is also a very convenient method to use for reducing the level of harmful pathogens such as Salmonella.

Pelleting has also been claimed to improve nutrient availability through higher nutrient digestibility, but this is a claim which lacks substantiation in poultry nutrition. However, the increased feed intake as a consequence of the pelleting process will improve feed efficiency, which is simply an effect of the increased weight gain and thus a lower maintenance requirement as a proportion of the total requirement. Stated using a simple example; when feed intake of a broiler chicken approaches zero, the feed/gain approaches infinite.

It is probably correct to state that at an early stage of universal adoption of the pelleting technology, very little attention were paid to the structure of the ingredients contained in the pelleted diet. In fact, it was even thought that a very fine grinding was beneficial. True, a fine grinding is a prerequisite for digestion to be efficient, as a large surface area will maximize contact between nutrient substrates and enzymes and other digestive components necessary for nutrient breakdown, and although the birds are particularly effective at reducing particle size through the wet abrasion grinding taking place in the gizzard, a reduced need for particle reduction in the gizzard would potentially save energy use which could then be used for growth.

In addition, the ability of the pellet to withstand stresses during handling and remain intact has always been a concern, and a fine grinding was thought to be necessary for a good pellet durability.

With the emergence of a plethora of research demonstrating the importance of a minimum level of coarse particles in the poultry diets, this view has changed dramatically (Svihus, 2011). The pellet will very quickly disintegrate in contact with water. Thus, the resulting particles contained in the pellet will determine the coarseness of the diet judged from a digestive tract perspective. This level of structure, which can be termed microstructure to distinguish it from the macrostructure defined by the size of the pellet, has a profound effect on digestive tract functionality, primarily through its gizzard-stimulating properties.

Thus, the perfect broiler diet has a macro-structure which assures that feed intake is not a limiting factor for performance, while at the same time containing a sufficient amount of structural components contained in the pellet micro-structure to assure that the gizzard is able to develop normally. This short overview will attempt to present the facts as far as we know them, while at the same time deal with the delicate balances and potentially conflicting interests emerging when micro- and macrostructure is attempted to be optimized. In addition, issues related to processing efficiency and cost, which are crucial factors in an industry with small profit margins, will also be dealt with when possible.

Grinding for A Coarse Microstructure

As stated above, it has been demonstrated beyond doubt that access to a minimum amount of structural components is necessary for a normal development of the gizzard. A poorly developed gizzard will not be able to carry out its many important functions. Due to a small holding volume which reduces retention time, nutrient digestibility will potentially be reduced, not the least due to the important moisturization process and the protein digestion facilitated by pepsin secreted by the proventriculus. The grinding action is also impaired, resulting in too large particles entering the small intestine (Hetland et al. 2002). In addition, it has been demonstrated that pH is less reduced, which also may affect the digestion process negatively. The digestion process in the small intestine may also be negatively affected, since the gizzard has an important role in acting as a pacemaker for feed flow into the small intestine. It has been demonstrated that feed material flows more rapidly into the small intestine in starved birds with an underdeveloped gizzard, resulting in a large amount of undigested nutrients in the ileum (Sacranie et al. 2017). Lastly, the heightened pH of a poorly functioning gizzard may also make the bird more susceptible for pathogenic microflora arriving through the oral route.

As sufficient amount of large particles in the diet may be achieved by just making sure that the feed ingredients are not too finely ground. Chiefly, it is the cereals in the diet that will contribute the most to diet microstructure. These raw materials are purchased as whole seeds and constitute a majority of the diet. Cereals such as oats and barley are particularly effective in stimulating gizzard development due to the hulls enchasing the seed. Thus, if the diet contains a sufficient amount of these cereals, a coarse grinding is not as critical. These tough fibre components are tougher to degrade in the grinding process, and thus will stimulate gizzard development even after rather fine grinding. However, these cereals are often not available at a cost-effective prize. With more typical maize- and wheat-based diets, a coarse grinding is imperative for a sufficiently good microstructure.

Cereals and other feed ingredients are most commonly ground using a hammer mill. In this grinding process, the feed ingredients fall into a chamber and are hit by hammers attached to a horizontal rotating axis. When the hammers hit the cereals, these are shattered into smaller particles. But the grinding process does not end here. To be able to pass out of the hammer mill, the particles will need to pass through holes in a screen surrounding the rotating hammers. Here, particles are further broken down to finer particles due to frictional forces before they are passing out of the holes, a process which needs to be facilitated by air suction. The diameter of the holes in the screen is usually between 3 and 9 mm, but the average particle size is much smaller due to the difficulty of the particles in being able to change direction and pass out of the holes. As an example, a screen with 3 mm holes will mainly produce particles with a particle size below 1 mm. Sometimes a roller mill is used. A roller mill grinds by crushing the feed particles through one or several pairs of rolls which rotates towards each other and where the distance between the rolls are small enough to crush the particles as they pass between the rolls. By adjusting the distance between the rolls, the roller mill will be able to produce very coarse particles.

If hulls-containing cereals are used, 10 to 20 % of these cereals will probably be sufficient for a stimulation of gizzard development. Thus, in such a situation, a coarse grinding is not critical. With maize- and wheat-based diets, however, the grinding must be coarse enough for the cereal particles to stimulate gizzard development. Although more research is needed, it is possible that the broiler diet should contain at least 30 to 40 % particles larger than 1 mm, of which half should be larger than 2 mm. When a hammer mill is used, this would mean that screen hole diameter as a rule of thumb should be at least 6

mm. Hammer mills vary widely, and the grinding result is dependent on a number of factors such as size, hammer tip speed and air suction. Thus, dry sieving of the ground material should always be carried out to figure out the right sieve and hammer mill adjustment, and this should be carried out separately for different diet compositions. Maize will for example not give the same particle size distribution as wheat at the same hammer mill settings. Dry sieving can be carried out using many different selections of sieves which are stacked atop of each other, with the coarsest sieves on the top. However, the most important in this context is to have at least two to three sieves 1 mm or larger, e.g. 1 mm, 2 mm and 3-4 mm.

If the grinding is too coarse, there may be negative effects on both feed intake and nutrient digestion, but the tolerance appears to be very high, at least in pelleted diets. Naderinejad et al. (2016) found no difference in performance of broiler chickens given pelleted maize-based diets hammer-ground through a 3, 5 or 8 mm screen. A number of other studies have confirmed that broilers have a very large capacity to grind coarse cereal particles.

Pelleting for A Coarse Macrostructure

Although a coarse grinding will result in a complete diet with many large particles which will result in a higher feed intake than if a finer grinding was used, even the most perfect coarsely ground diet will not be able to give a maximized feed intake. Thus, pelleting is usually carried out.

The pelleting process starts with addition of around 4 % water in the form of saturated steam in the conditioner. During the very short time (usually less than a minute) the feed remains in the conditioner, the paddles are mixing the feed with the steam so that the feed takes up the water and heats up to around 70 – 80 ºC. This facilitates the pellet formation in the pelleting process. The conditioned mash is falling into a chamber formed by a rotating short cylinder, which is perhaps 1 m in diameter and 30 cm long. This short cylinder is the pellet die, and forms the pellet when the feed is forced through the numerous holes drilled radially through the cylinder. The feed is squeezed into the hole by one to three rolls running along the inner diameter. Once the feed exits the die on the outside, fixed knifes cuts/breaks the pellet into a length which among others are dependent on distance between the knife and the die. Finally, the pellet is cooled and dried by ambient air blown through the feed, resulting in a feed with approximately the same water content as before conditioning.

The particles in the pellet are not very strongly bound together, and particularly the ends of the pellet have a tendency to be eroded during transport. Thus, there will always be particles smaller than the pellet in a pelleted diet. The durability test is used to assess how well the pellet can withstand stress, and is a very much used measure of pellet quality. The tumbler test and the Holmen tester are the two most used methods, and although they do not produce the same durability, they usually rank diets similarly. They are both designed on the principle of moving the pellets around in a chamber for a given period of time, after which the pellet is sieved through a sieve slightly smaller than the diameter of the pellet. Pellet durability is expressed as a figure between 0 and 100, and indicates the percentage intact pellets after the durability test. In countries where a lot of wheat is used, a durability above 90 is often obtained, while for maize-based diets, durability is often at least 10 to 20 percentage points lower. The minimum amount of intact pellet required for broilers to not observe a reduced feed intake will vary dependent of a number of factors, but the few experiments carried out indicate that birds can tolerate at least 15 % fines in the diet at the feeder, indicating that a durability as low as 85 (measured using the Holmen test) would not compromise feed intake. In some situations, they certainly will be able to cope with considerably lower durability without any reduced performance.

Given that we have succeeded with the pelleting process and that a large portion of the pellets remain intact all the way to the feeder, the question arises what the ideal diameter and length of the pellet should be. First, this will certainly depend on the age of the bird. So to deal with this question, we probably should deal with this question separately for birds below 10-14 days of age and birds older than that. The effect of varying pellet length seems to not have been studies extensively. In regards to diameter, however, crumbled (i.e. the pellet is crushed through a pair of rolls) 3 to 4 mm pellets or a 2.5 mm pellet is commonly used in the starter period. However, the few experiments carried out to test what the effect of varying macrostructure of the starter diet, seems to indicate that broiler chickens below the age of 10 to 14 days are not sensitive to pellets with a diameter of at least 3 to 4 mm. Rubio et al. (2020) found no difference in performance of broilers given either a 3.3 mm diameter pelleted diet or the same diet crumbled. Even more surprising was the results by Lemon et al. (2019). Even a 4.74 mm diameter pellets crumbled so gently that 75 % of the pellets were still intact did not result in poorer feed intake than more completely crumbled pellets at 7 and 14 days of age. These observations of a high tolerability of large particles even in day-old birds fits with the classical data of Portella et al. (1988). Here, diet selection studies were carried out on broiler chickens at different ages throughout the growth period. Even as early as 8 days of age, birds preferred the largest particles larger than 2.36 mm, followed by particles larger than 1.18 mm.

Not surprisingly, Portella et al. (1988) observed that the preference for large particles increased with age. Thus, birds older than 10 days can cope with particles with a much larger diameter than 3 mm. Several rather recent experiments using wheat- or maize-based diets have demonstrated that broiler chickens performed just as well when the pellet diameter was 4.4 to 4.76 mm as when the diameter was 3 mm (Abdollahi et al. 2013a,b; Singh & Ravindran 2014; Rubio et al. (2020). When it comes to pellet length, few experiments appears to have been carried out, as mentioned above. However, Abdollahi et al. (2013a,b) found no negative effect on performance when a 3 mm or 4.76 mm diameter pellet was approximately doubled in length from 3 to 6 mm by increasing the distance between the knife and the die. In fact, for the wheat-based diet, the longest pellets significantly increased weight gain and improved feed/gain for the 4.76 mm diet. Thus, it appears that even pellets with a diameter as large as 4.76 mm can be at least 6 mm long without negative effects on feed intake. Although more research is needed, it is possible that due to the way birds grasp and swallow pellets, combined with the pecking activity towards the pellet, the pellet can be considerably longer than the diameter without reducing feed intake.

Thus, it appears that just like the story about the microstructure, the dimensions of the pellet can be larger at least than what is commonly used in Europe. In other words, there appears to be room for increasing macrostructure in many broiler operations.

Room for A Coarser Micro- and Macrostructure of Broiler Diets

In a Master thesis work at our university carried out with the kind support of Aviagen, 54 broiler diets (excluding starter diets) from all over the world, selected to give a representative picture of global broiler diet structure, were assessed (Wang, 2021). Around half the diets had a diameter lower than 3.5 mm, and only 7 had a diameter above 4 mm. Average length was around 6 mm, but with large variation both between diets and between individual pellets within diet. The microstructure was also measured. When diets are pelleted, dry sieving will not give a measure of microstructure, but rather the more laborious wet sieving method must be used. Here, the pelleted diet is first soaked in water, followed by washing the dissolved diet through a series of sieves. After drying the contents on each sieve, the particle distribution can be calculated. On average, the 54 diets contained 28 % particles larger than 1 mm, and 17 % particles larger than 2 mm. Although this is not a very disappointing microstructure, only 9 diets had 40 % and 20 % or more particles larger than 1 mm and 2 mm, respectively. Thus, this Master thesis clearly indicate that there are room for increasing both micro- and macrostructure of commercial broiler diets.

The benefits of increasing microstructure is primarily the beneficial nutritional effects related to digestive tract functionality. However, a coarser grinding would also increase grinding capacity and reduce energy consumption. In an unpublished experiment recently carried out at Centre for Feed Technology at Norwegian University of Life Sciences, there was a 43 and 59 % reduction in energy consumption per ton when the 3 mm sieve in the hammer mill was replaced with a 8 mm sieve for maize and wheat, respectively (i.e on average about a halving of energy consumption per ton). For the harder-to-grind hulls-containing cereals barley and oats, the saving in energy use was even larger, at 64 and 78 % reduction in energy use, respectively. Since many mills are set at a certain amperage, this means that grinding capacity increases proportionally as energy use is reduced.

For pelleting on the other side, the main advantage of increasing pellet diameter lies in the considerable savings in energy consumption. In an unpublished experiment recently carried out with a coarsely ground wheat- and maize-based diet at Centre for Feed Technology, there was a 38 % reduction in energy consumption per ton feed in the pelleting process (at similar conditioning) when a 3 mm die was replaced with a 5 mm die (diameter/effective length ratio approximately the same between the dies). As the pelleting process is the most energy-demanding step in feed production, such a large reduction in energy consumption has a significant economic effect. Also, just as for the milling process, capacity will also increase tremendously.

But it is not only lower energy consumption and higher pelleting capacity which would be benefits of a larger pellet diameter. A larger pellet diameter would also facilitate a coarser microstructure. This is because the pelleting process not only shapes the ground feed into pellets, it also has a considerable grinding effect as the particles are squeezed into the holes in the die by the rolls (Svihus et al. 2004; Vukmirovic et al. 2017). The large particles are particularly prone to grinding in the pelleting process. In an experiment where wheat-based diet coarsely hammer-milled through a 6 mm screen was pelleted using a 3 mm die, more than half the particles larger than 2 mm disappeared in the pelleting process (Svihus et al 2004). When the diet was very coarsely ground using a roller mill, 80 % of the particles larger than 2 mm disappeared in the pelleting process. With a larger pellet diameter, it is logical that this unfortunate grinding effect is reduced. Although firm data are scarce, Singh and Ravindran (2015) did wet sieving of diets pelleted through a 3 and a 4.76 mm die. Particles larger than 1 or 2 mm increased considerably when the largest die diameter was used. When whole wheat was added prior to pelleting, the 4.76 mm die had more than the double amount of particles larger than 2 mm compared to the 3 mm die. Thus, an additional advantage of using a larger pellet diameter, is that a larger proportion of the coarsely ground ingredients remain intact after pelleting.

A persistent worry when pelleting diameter is increased is that pellet durability may be compromised. This is a valid concern, as many experiments have demonstrated that decreasing pellet diameter increases durability. However, quite surprisingly, in fact two of the papers referred to above where broiler diets pelleted through a 3 mm or a 4.76 mm die were compared had a similar durability independent of die diameter (Abdollahi et al. 2013a; Singh and Ravindran, 2015). In one of the experiments where the diet was maize-based, the durability was reduced when the 4.76 mm die was used (Abdollahi et al. 2013b), but the reduction was only of a commercially significant magnitude for one of the two knife distances used. In addition, it did not have any effect on broiler performance. Another experiment neither found a consistent reduction in pellet durability when pelleting diameter increased from 2.5 to 4 mm for different diet compositions (Moradi et al. 2019).

It should also be mentioned that a number of factors will affect durability of pellets. The effective length of the hole (i.e the length from inner diameter to out diameter of the die, minus counterbore if present) will be important, where a lower compression with higher diameters can be compensated for by increased effective length. Feed pelleted through a larger die can also be conditioned to a higher temperature, which also would increase pellet durability. Thus, a larger diameter surely does not necessary result in a poorer pellet durability.

Conclusion

It seems clear that both the micro- and the macrostructure of pelleted broiler diets may have a potential to be increased compared to common practices. The ingredients can be ground using 8 to 9 mm diameter of the holes in the hammer mill screen, and the pellet diameter can probably be increased to around 5 mm. Such a diet will result in considerable savings in production costs, both due to increased capacity and a reduced energy consumption per ton feed produced. In addition to a more cost-effective production process, the coarser grinding and the lower extent of grinding in the pellet press with a larger pellet diameter, will potentially improve performance and health in broiler production.

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OP[38] Effect of Non-Ionic Emulsifier on Energy Saving During Pellet Production as well as Feed Formulation and Growth Performance of Broilers

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Feed Industry is facing lot of challenges in current scenario of Covid-19 outbreak and thereafter, these include disease challenges like African Swine Fever in pigs, Avian Influenza in poultry; high prices of raw material, essential ingredients; logistic issues, shipment delays; feed mill efficiencies, bringing the efforts to reduce the cost of production and most importantly to consider the sustainability factor as the growing demand from consumers to look for green products with low carbon footprint. High electricity costs and even the energy crises worldwide making it difficult for feed mill operations as the pellet production is using the highest power consuming motors to run the pellet mill.

Nutritionists usually ignore the fact that poor feed processing is directly affecting the nutritional value of feed and ultimately animal performance. Emulsifier is unique in character which works both in feed processing and at animal level to improve digestibility and feed efficiency. In general, a higher PDI means an animal has an increased resting period as it must spend less energy consuming the pellets or picking up smaller particles. This helps the animal conserve energy, and the caloric value of the feed is enhanced compared to feeds requiring a higher level of energy to digest.

Energy and protein are two key sources that have substantial contributions to production performance and economics. Efficient utilization of these two components can give better production and profit. The feed contains water-insoluble components like vegetable proteins, fats/oils and fat-soluble vitamins which all are affected positively by emulsifiers for increased digestion. As a result, lower energy diets can be formulated for broilers with the same performance, with much lower feed cost and more economical and sustainable production.

The proteins have a tightly folded conformation which restricts the access of the hydrolytic enzymes to their substrates. Emulsifiers opens this structure, making it more accessible. Emulsifier has proven to increase the protein digestibility and hence reducing the Nitrogen excretion in poultry. Emulsifier has impact on environment as it reduces the Nitrogen / Ammonia in environment as it helps to digest Protein efficiently. More digestibility, less wastage, less release of Ammonia in environment.

A trial was conducted at the Department of Animal Nutrition, POZNAN University of Life Sciences, Poland to evaluate the effect of Non-ionic emulsifier (Bredol) in diets with reduced AME, on broiler performance and digestibility. The trial was carried out with 600, 1-day-old male Ross 308 chicken broilers in 6 treatments (Positive Control, Negative Control – 60 kcal, Negative control – 100 kcal, supplemented or not with 400 ppm/kg), with 10 replicates per treatment and 10 birds per replicate. The total treatment duration was 42 days with a 3-phase feeding system (starter 1-10d), grower (10-24d), and finisher (24-till end). For the determination of digestibility, finisher feed was supplemented with TiO2 (dose: 0.3%) as an indigestible marker. On day 21, collection trays were installed in floor pens for excreta collection. After approximately, 4 h, 10 samples per treatment, free from contamination from feed and feathers, were collected. The experiment was conducted as a completely randomized design and growth performance was analyzed by multiple analyses of variance (MANOVA) using the general linear model's procedure of R environment.

Results show that there were no differences in BWG, FI, and FCR across treatments throughout the first 10 days of the trial. From day 10th to 21st there were no differences in BWGs. There was no interaction between Emulsifier inclusion and dietary energy level. Although FI increased when dietary AMEn decreased ($P<0.05$), the Emulsifier inclusion decreased FI ($P0<0.05$). The FCR increased significantly ($P<0.05$) when AME was reduced by 100 kcal/kg.

During the finisher period, the use of the Emulsifier improved BWG ($P<0.05$). Likewise in the grower period, AMEn reduction increased FI ($P<0.05$). There was an interaction ($P<0.05$) between AMEn reduction and Emulsifier inclusion in FCR. AMEn reduction didn't increase FCR when Emulsifier was used.

Considering the whole trial, Emulsifier inclusion improved BWG irrespective of AMEn reduction ($P<0.05$). Feed intake increased when dietary AMEn was reduced. There was a tendency to reduce FI after Emulsifier inclusion. Energy reduction increased FCR ($P<0.05$) however Emulsifier inclusion reduced it ($P<0.05$), and this resulted in interaction ($P<0.1$). Weight-corrected FCR (WtC FCR) was improved after Emulsifier inclusion (1.44 vs. 1.384). There was a tendency to increase WtC FCR after dietary AMEn reduction ($P<0.1$), but changes were insignificant for Emulsifier-supplemented treatments.

The use of Emulsifier improved N retention ($P<0.05$), ether extracts total tract digestibility ($P<0.05$) as well as AMEn. Dietary energy reduction affected AMEn from 3130 to 2980 kcal/kg ($P<0.05$). There was no effect of dietary energy reduction on ether extract digestibility.

From the results, we can conclude that Emulsifier inclusion in feed can improve protein utilization and thus can reduce protein cost and ammonia production in the air from fecal matter. We can also get better FCR and body weight gain even by reducing the energy level up to 100 Kcal/kg and controlling feed intake, which will also reduce feed energy costs. Significant improvement in Nitrogen retention clearly shows the direct positive impact on environment and this emulsifier is good choice for considering sustainability factor.

OP[39] Effects of Supplementing a Combination of Lysolecithin, a Synthetic Emulsifier and Monoglycerides in Dry and Liquid Form in High and Low Energy Diets on Performance and Welfare of Broiler Chickens

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Abstract

With high production costs and growing concerns on animal welfare, the poultry industry is in need for solutions that reduce feed costs without impairing performance, while keeping welfare to the highest standards. Supplementation of a combination of lysolecithin, a synthetic emulsifier and monoglycerides (LEX) in liquid and dry form to broiler diets with different energy levels was investigated to determine their effect on performance, litter quality and subsequent occurrence of footpad lesions. 1248, day-old Ross 308 broilers were assigned to one of six treatments for a 42-day study: a control basal diet with a high energy content (HE); HE + 300g/t LEX in liquid form; HE + 500g/t LEX in dry form; a control basal diet with a low energy content (LE, -90 kcal/kg respect to HE), LE + 300g/t liquid LEX and a LE + 500g/t dry LEX. Each treatment consisted of 13 pens (replicates) of 16 birds each. Feed intake and weight were measured on days 0, 10, 21 and 42. On day 42 a litter sample was collected from each pen (5 sites within the pen) and two birds per pen were assessed for footpad lesions. Results showed a higher ($p<0.05$) cumulative bodyweight gain (BWG) with LEX supplementation. However, there was no effect ($p<0.05$) of energy level or interaction between energy and supplementation. An increased ($p<0.05$) feed intake was observed for the LE diets, however the cumulative feed efficiency (FCR) of the LE + LEX treatments remained equal ($p>0.05$) to the HE Control, which suggests LEX application allowed the birds to cover the energy gap. The cumulative FCR of the LE diets were increased ($p<0.05$) compared to the HE, however there was a tendency ($p<0.1$) towards improved FCR when LEX was used. A significantly improved dry matter percentage was found in the litter of birds fed LEX, compared to the control HE and LE groups. This reflected into lower ($p<0.05$) occurrence and severity of footpad lesions in birds supplemented with LEX compared to the two control treatments. In conclusion, feeding broilers a combination of lysolecithin, a synthetic emulsifier and monoglycerides resulted in improved bird performance. Adding LEX to LE diets resulted in same BWG as the HE Control. The use of the LEX also improved litter quality and footpad health therefore improving animal welfare.

Keywords: Lysolecithin, digestibility, amino acids, fatty acids, nutrient absorption

Introduction

The major factor influencing differential utilization of nutrients and growth performance in broilers is dietary energy level [1], and reductions in energy density are correlated with impaired growth performance [2, 3, 4, 5]. Meeting the high energy and amino acid requirements of broilers from fast-growing genetics has been proven to result in better feed efficiency [6], and involves formulating diets with highly energetic and proteinaceous, and very often expensive, feed ingredients. This is of particular concern considering that feed represents around 70% of the total production cost of broiler chickens [7]. Keeping the balance between broiler growth performance and production profitability is not an easy task for the modern poultry industry, and the sustained rise of in the cost of feed raw materials remains a primary challenge [8], especially in an increasingly volatile and disrupted global supply chain environment. with the multiple disruptions created by COVID-19 pandemic in the supply chain. Maximizing the utilization of nutrients, such as energy and amino acids, at the lowest possible feed cost, can offer nutritionists a window of opportunity to improve overall broiler profitability. Reformulating diets to lower cost and nutrient density with the use of lysolecithin based combinations has proven to be an effective strategy in this respect [3, 5, 9-12]. When birds get into contact with litter high in moisture and ammonia content, contact dermatitis can occur, resulting in lesions on the foot pads and ammonia burns to the breast of birds. This can result in substantial downgrading of carcasses and even condemnation for human consumption, with obvious economic implications [13]. However, there is scarcity of data on the effect of lysolecithin based combinations in footpad lesions and breast burns mitigation. Therefore, a study was performed to, beyond assessing the impact on performance of applying a combination of lysolecithin, a synthetic emulsifier and monoglycerides (LEX), in different physical form and in a high and low energy diet, also evaluate the effect

on food pad health and breast burn scores.

Material and Methods

The trial was conducted at Roslin Nutrition (Scotland). All experimental procedures were in line with commercial practices and were compliant with all local animal welfare legislation. The duration of the study was 42 days. A total of one thousand two hundred and forty-eight one-day-old male Ross 308 broilers (43.6 g at hatch) were randomly allocated to 6 dietary treatments consisting of 13 pens of 16 birds each: a control basal diet with a high energy content (HE); HE + 300g/t LEX in liquid form; HE + 500g/t LEX in dry form; a control basal diet with a low energy content (LE, -90 kcal/kg respect to HE), LE + 300g/t liquid LEX and a LE + 500g/t dry LEX. Diets were fed in 3 phases: a starter diet from 0 to 10 days; a grower diet from 11 to 21 days and a finisher diet from 22 to 42 days (Table 1). Nutrient values of the diets were calculated according to the CVB 2018 values of feedstuffs [14]. The combination of lysolecithin, a synthetic emulsifier and monoglycerides (LEX) used in this study was LYSOFORTE® Extend and was supplied by Kemin Europa NV, Herentals, Belgium.

All birds were weighed at their arrival from the hatchery and at days 10, 21 and 42. Feed bags, as well as feed remaining in the feeders, were weighed at the same time to calculate feed intake and feed conversion ratio (FCR). Pens were monitored daily for mortality. At day 42, two birds per pen were assessed for footpad scores and breast burn scores according to a standardized evaluation system: 0 = no evidence of damage; 1 = mild damage; 2 = severe damage. Additionally, litter was collected from each pen and analysed for dry matter (DM) content.

All data were statistically analyzed in the Fit Model platform of JMP 16, with means separation achieved using Tukey's HSD. $P<0.05$ was taken to indicate significant difference.

Results and Discussion

Table 2 presents the effect of supplementing LEX in dry or liquid form to high or low energy diets on growth performance of in broilers. Growth performance was a bit lower compared to Ross 308 male performance objectives [15] for the same growing period of 42 days. We hypothesized that the reason behind it could have been the lower feed intake compared to that of the performance objectives. Cumulative feed intake per period were on average 18g, 99g, 162 g and 288 g lower for 0-10 days, 11-21 days, 22-42 days and 0-42 days periods respectively compared to Ross 308 male performance objectives [15].

By day 10, broilers fed the supplementation of LEX increased ($p<0.05$) the body weight (BW) of the birds when applied to both high and low energy diets. During days 11-21, the broilers fed HE diets exhibited higher ($p<0.05$) BWG, FI and better ($p<0.05$) FCR, compared to broilers fed LE diets. During day 21-42, birds fed LEX in liquid and dry form, either supplied in a HE or LE diets, again showed higher ($p<0.05$) BWG compared to non-supplemented broilers, with LEX liquid achieving the highest values in BWG compared to LEX dry for the same energy level. This pattern was reflected in better ($p<0.05$) FCR following both LEX dry and liquid supplementation for the same growing period. The cumulative results (0-42 days) showed that broiler fed HE exhibited better ($P<0.05$) FCR than broilers fed LE diets. From 0 to 42 days, LEX supplementation in dry and liquid form improved ($p<0.05$) BWG and tended to improve ($p<0.1$) FCR when applied to both HE and LE diets, with the liquid form delivering the best results.

Table 1. Ingredients and calculated nutrient composition of the experimental diets (% as-fed)

	Starter (0-10 days)		Grower (11-21 days)		Finisher (22-42 days)	
Ingredients (%)	**HE**	**LE**	**HE**	**LE**	**HE**	**LE**
Corn	46.88	45.70	48.73	49.31	51.81	52.31
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Rapeseed meal 35% CP	-	3.50	-	4.23	-	4.33
Soybean meal 47% CP	35.27	35.21	32.49	31.05	30.43	28.63
Full-fat soya 35% CP	1.50	-	2.40	-	2.00	-
Soya oil	2.40	1.80	3.00	2.10	3.14	2.20
MCP	0.92	0.88	0.68	0.65	0.45	0.42
Sodium Bicarbonate	0.30	0.28	0.18	0.19	0.10	0.10
Limestone	1.19	1.17	1.18	1.13	0.90	0.85
Sodium chloride	0.27	0.27	0.30	0.29	0.30	0.30
DL-Methionine	0.333	0.30	0.290	0.271	0.227	0.207
L-Lysine HCl	0.233	0.208	0.144	0.166	0.084	0.106
L-Threonine	0.116	0.097	0.082	0.083	0.040	0.040
L-Valine	0.076	0.054	0.019	0.021	-	-
Phytase 5000	0.010	0.010	0.010	0.010	0.010	0.010
Vit-Min Premix[1]	0.500	0.500	0.500	0.500	0.500	0.500
Nutrients						
AME (kcal/kg)	2885	2795	2960	2860	3010	2910
Crude Protein (%)	22.50	22.50	21.00	21.00	20.00	20.00
Fat (%)	5.06	4.25	5.85	4.64	6.00	4.81
Crude Fibre (%)	3.20	5.63	3.14	3.45	3.06	3.38
Dig. Lysine (%)	1.22	1.22	1.10	1.10	1.00	1.00
Dig. Met (%)	0.63	0.61	0.57	0.56	0.50	0.49
Dig Met + Cys (%)	0.90	0.90	0.84	0.84	0.76	0.76
Dig. Thr (%)	0.79	0.79	0.73	0.73	0.66	0.66
Dig. Ile (%)	0.82	0.83	0.78	0.77	0.75	0.73
Dig. Val (%)	0.96	0.96	0.87	0.87	0.82	0.81
Dig. Arg (%)	1.34	1.36	1.28	1.25	1.21	1.19
Dig. Trp (%)	0.23	0.24	0.22	0.22	0.21	0.21
Calcium (%)	0.95	0.96	0.90	0.90	0.75	0.75
Total P (%)	0.58	0.60	0.52	0.54	0.46	0.48
Dig. P (%)	0.45	0.45	0.40	0.40	0.35	0.35
Sodium (%)	0.19	0.19	0.17	0.17	0.15	0.15

[1]Provided per kilogram diet: Vitamin A: 12,000 IU; Vitamin D3: 2,400 IU; Vitamin E: 30 mg; Vitamin K3: 3 mg; Vitamin B1: 2.2 mg; Vitamin B2: 8 mg; Vitamin B6: 5 mg; Vitamin B12: 11 µg; Folic acid: 1.5 mg; Biotin: 150 µg; Calcium pantothenate: 25 mg; nicotinic acid: 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0.33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.15 mg; Ethoxyquin: 150 mg, xylanase: 45,000 U.

Table 2. Effect of supplementing LEX in dry or liquid form to high or low energy diets, on growth performance of in broilers.

	High Energy			**Low Energy**			**SEM**	**P-value*** **		
	Control	LEX DRY	LEX LIQUID	Control	LEX DRY	LEX LIQUID		Energy	LEX	Energy*LEX
BW* day 0	43.44	43.64	43.88	43.49	44.15	43.01	0.4061	0.7759	0.4970	0.2730
BW day 10	261	268	273	264	271	276	3.2545	0.2842	0.0034	0.9941
BW day 21	908	915	941	910	912	908	7.9126	0.0960	0.1671	0.0934
BW day 42	2766	2873	2938	2761	2831	2936	61.028	0.8007	0.0332	0.9269
BWG* day 0-10	217	225	233	221	227	230	3.1668	0.2559	0.0026	0.9877
BWG day 10-21	647	647	668	646	640	632	7.8407	0.0352	0.7417	0.0977
BWG day 21-42	1853	1958	1997	1855	1920	2027	58.075	0.9714	0.0435	0.8559
BWG day 0-42	2718	2829	2895	2722	2787	2893	60.994	0.8021	0.0331	0.9242
FI* day 0-10	278	270	285	266	278	283	5.4071	0.6302	0.0863	0.2257
FI day 10-21	902	900	916	908	934	937	9.8896	0.0217	0.1220	0.3790
FI day 21-42	3298	3375	3410	3411	3424	3563	61.568	0.0529	0.1319	0.7230
FI day 0-42	4479	4545	4611	4584	4636	4783	67.771	0.0400	0.0737	0.8376
FCR day 0-10	1.279	1.203	1.245	1.203	1.223	1.215	0.0201	0.1062	0.4147	0.0851
FCR day 10-21	1.394	1.392	1.373	1.405	1.460	1.485	0.0174	<.0001	0.2256	0.0287
FCR day 21-42	1.784	1.726	1.710	1.874	1.786	1.765	0.0343	0.0237	0.0356	0.8719
FCR* day 0-42	1.649	1.607	1.595	1.698	1.664	1.656	0.0208	0.0027	0.0747	0.9560
Adj. FCR 2.8 kg***	1.657	1.591	1.564	1.707	1.657	1.623	-	-	-	-

*BW: body weight (g/bird); BWG: body weight gain (g/bird); FI: feed intake (g/bird); FCR: feed conversion ratio (kg of feed/kg of BWG)

** n= 13 replicates per treatment (16 birds per replicate)

***Adjusted FCR to 2.8 kg of BW according to Ross Broiler Pocket Guide [16]

The effect of LEX supplementation in FCR when applied to NE and LE diets became more relevant when adjusting FCR for the same final BW of 2.8 kg [16], being 66 and 93 g feed/kg BWG lower for LEX Dry and Liquid respectively compared to HE Control and 50 and 84 g feed/kg BWG lower for LEX Dry and Liquid respectively compared to HE Control. When comparing the NE control group with the LE control group, findings showed that lowering the AME level with 90 kcal/kg in starter and 100 kcal/kg in grower and finisher phase (LE Control), clearly impaired feed efficiency with 50 g of feed/kg BGW (1.707 LE Control vs. 1.657 HE Control). The use of LEX dry in LE diets entirely compensated this impact ensuring the FCR remained the same as the HE Control (1.657). The FCR even improved when using LEX Liquid (1.623 LE LEX Liquid vs. 1.657 HE Control).

Table 3 presents the effect of supplementing LEX in dry or liquid form to high or low energy diets on dry matter of the litter. The addition of LEX in both HE and LE diets, reduced ($p<0.0001$) litter moisture compared to the control diets. This confirms observational evidence from the trial, where litter in the supplemented pens was visibly better than in the control pens.

Table 3. Effect of supplementing LEX in dry or liquid form to high or low energy diets, on litter DM at 42 days.

	High Energy			**Low Energy**			**SEM**	**P-value***		
	Control	LEX DRY	LEX LIQUID	Control	LEX DRY	LEX LIQUID		Energy	LEX	Energy*LEX
Litter DM, %	50.63^{b}	68.13^{a}	66.33^{a}	49.44^{b}	64.32^{a}	66.10^{a}	2.019	0.2927	<0.0001	0.6574

* n = 13 replicates per treatment

When footpad lesions and breast burns between treatments were assessed (table 4), the use of both LEX in dry and liquid form, regardless the dietary energy level, resulted decreased ($p<0.0001$) in the incidence and severity of lesions as seen in up to almost 68% and 86% reduction in foot pad lesions and breast burns respectively. These reductions are likely linked to the reduced litter moisture seen with LEX-supplemented diets.

Table 3. Effect of supplementing LEX in dry or liquid form to high or low energy diets, on food pad score and breast burns at 42 days.

	High Energy			Low Energy			SEM	P-value
	Control	LEX DRY	LEX LIQUID	Control	LEX DRY	LEX LIQUID		
Food pad score*	1.65[a]	0.65[b]	0.69[b]	1.73[a]	0.88[b]	0.77[b]	0.0098	<0.0001
Breast burns*	1.65[a]	0.92[b]	1.12[b]	1.77[a]	1.00[b]	0.88[b]	0.1173	<0.0001

*0 = no evidence of damage; 1 = mild damage; 2 = severe damage.

** n =13 replicates per treatment (2 birds per replicate)

Conclusions

The use of HE diets in broilers resulted in improved FCR compared to the use of LE diets. Supplementing LEX in dry or liquid form to HE or LE diets improved final BW and tended to reduce FCR, while improving bird welfare through reduced incidence and severity of food pad lesions and breast burns.

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OP40 Effects of the Dietary Zinc Source and Vitamin E Level on Carcass Yield and Meat Quality in Distressed Broilers

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Abstract

The objective of this study was to evaluate the effect of the interaction of the zinc source (ZnSO4 vs. zinc amino acid complex) and vitamin E level (50 IU/kg vs. 100 IU/kg) on meat yield and quality in broilers subjected to chronic cyclic heat stress in the finisher phase. A total of 1224 one-day-old male Ross 308 broilers were randomly distributed among four dietary treatments. Each treatment contained nine replicates of 34 birds, housed in floor pens in a temperature- and lighting-controlled room. Treatments were organized in a 2 × 2 factorial arrangement: two sources of zinc, 60 mg/kg of Zn as $ZnSO_4$ or 60 mg/kg of Zn as zinc amino acid complexes (ZnAA), combined with two levels of vitamin E (50 or 100 IU/kg). From day 28 until day 37 (finisher phase), all birds were subjected to chronic cyclic heat stress (32 ± 2°C for 6 h daily). In the present study, it was observed that replacing $ZnSO_4$ with ZnAA increased breast meat weight and yield of broilers reared under chronic cyclic heat stress conditions, whereas total slaughter yield was not affected. Moreover, it was observed that replacing $ZnSO_4$ with ZnAA resulted in breast meat with a lower drip and thawing loss and a higher marinade uptake. In conclusion, replacing $ZnSO_4$ with more readily available ZnAA can improve breast meat yield and increase the water-holding capacity of breast meat of broilers exposed to chronic cyclic heat stress at the end of the production cycle. However, as no thermoneutral group was included in the present study, the observed effects of the zinc source cannot be generalized as a solution for heat stress. Moreover, the beneficial effects of ZnAA on breast meat yield and quality seem to be independent of the vitamin E level, and increasing vitamin E level has no additional beneficial effects.

Introduction

Heat stress is a major concern in poultry production because it has a profound effect on animal health and performance. Modern broiler breeds display reduced heat tolerance because of a lack of sweat glands and the high metabolism associated with low feed conversion and rapid growth [1,2]. Moreover, chronic heat stress leads to the deterioration of meat quality by changing the aerobic metabolism and by increasing glycolysis and fat deposition [3,4,5]. Consequently, the meat from broilers reared under high environmental temperatures is characterized by a pale color, low water-holding capacity (WHC), and therefore also increased cook and drip losses [5]. The impaired WHC is detrimental to the valorization of broiler meat which is further processed by marination, tumbling, and cooking [6]. Supplementation of vitamin A, C, and E can improve heat tolerance ability and animal performance during heat stress [7,8,9]. Some antioxidant minerals, including chromium [10,11], selenium [12], and zinc [13,14] are also used to prevent negative effects of heat stress. Zinc is an essential component of many enzymes, and it has both structural and catalytic functions in metalloenzymes. Furthermore, zinc is required for normal immune function as well as proper skeletal development and maintenance [13]. One of the most important functions of zinc is its antioxidant role and its participation in the antioxidant defense system. An increased level of reactive oxygen species is one of the main causes of decreased meat quality due to heat stress [6]. In broiler diets, $ZnSO_4$ and ZnO are two of the main inorganic zinc sources. There are also organic zinc sources available that are characterized by improved bioavailability [15,16]. A more readily available zinc source might be more efficient in reducing the adverse effects of stressors in broiler production, such as heat stress. Both zinc and vitamin E are frequently used antioxidants to alleviate the negative impact of heat stress. To the best of our knowledge, there is no information available concerning the interaction of zinc sources, as opposed to different zinc levels, on meat quality of broilers subjected to a temperature challenge, and on the

interaction with the vitamin E level. Therefore, the objective of this study was to evaluate the effect of the interaction of the zinc source ($ZnSO_4$ vs. zinc amino acid complex) and vitamin E level (50 vs. 100 IU) on meat quality and yield of broilers exposed to chronic cyclic heat stress in the finisher phase.

Materials and Methods:

Experimental design, animals, and diets : All experimental procedures in this study were in compliance with the European guidelines for the care and use of animals in research [17] and were approved by the Ethical Committee of the Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium, under authorization number 2017: 308.

A study was conducted using 1,224-day-old broilers in a 2x2 factorial arrangement of treatments with 9 replicate pens with 306 birds per treatment. study duration was 36 d. Birds were placed under cyclic HS from d 28 to 36 (32°C ± 2°C, 55 to 65% relative humidity, 6h daily).

Dietary treatments as illustrated in table 1 designed with two sources of zinc (Zn). Treatments contained equal amounts of elemental zinc (60 mg/kg), either originating from $ZnSO_4$ ($ZnSO_4.7H_2O$; containing 22% of elemental zinc, Sigma-Aldrich, St. Louis, United States) or originating as zinc amino acid complexes (ZnAA; containing 10% of elemental zinc, Availa®Zn, Zinpro Corporation, Eden Prairie, United States), and two levels of vitamin E (50 or 100 IU/kg; dl-α-tocopheryl acetate). The dietary treatments were provided in a wheat–rye based diet. Availa®Zn is a zinc chelate based on single amino acids from hydrolyzed soy protein and zinc bound in a one-to-one molar ratio.

Table 1. Different dietary treatment for broilers.

Dietary treatment	Zinc Source	Vitamin E
	60 ppm	IU/kg
ZnSO4-50VE	ZnSO4	50
ZnAA-50VE	Availa-Zn	50
ZnSO4-100VE	ZnSO4	100
ZnAA-100VE	Availa-Zn	100

ZnAA, zinc amino acid complex; Vit E, vitamin E, dl-α-tocopheryl acetate.

Slaughter yield and meat quality analysis : On day 37, three broilers per pen were selected, and live weight was determined by weighing the animals on a scale suited for animal use before transport to the slaughterhouse, where they were commercially slaughtered. Carcasses were immediately chilled after processing. Slaughter yield was determined approximately 24 h after slaughter (108 birds in total, 27 from each treatment group). The broilers were manually dissected by trained personnel to determine carcass, wing, leg (thigh and drumstick) and breast meat weight, and total yield. Carcass yield was calculated as eviscerated carcass weight relative to live weight before slaughter. The breast, thigh, drumstick, and wing yields were calculated as their weight relative to eviscerated carcass weight.

The different meat quality parameters were determined using the breast (pectoralis major muscles). Left breast fillets (n = 9 per treatment) were weighed, and color measurements were performed using a MiniScan EZ colorimeter (Hunterlab, Reston, VA) to record CIE L* (lightness), a* (redness), and b* (yellowness) values. Temperature and pH ultimate were measured using a Portamess® 910 (Knick, Berlin, Germany). The remaining left breast fillets (n = 18 per treatment) were vacuum-packed and transported to the University of Bologna (Italy, Cesena, Department of Agricultural and Food Sciences) in order to determine marinade uptake. The right breast fillets (n = 18 per treatment) were removed from the carcass and put in a polypropylene bag, hung for 24 h at 4 ± 2°C, and then blotted dry, and weighed again to measure drip loss. Drip loss was calculated as the difference between the weight before storage (W1) and the weight after storage (W2) relative to the weight before storage. Drip loss was calculated using the following formula: drip loss (%) = [(W1–W2)/W1] × 100. The remaining right (n = 9 per treatment) breast fillets were weighed (W3), vacuum-packed, and stored at -20°C for 4 days. They were then defrosted for 24 h at 5°C, blotted dry, and weighed (W4) in order to determine thawing loss. Thawing loss was calculated using the following formula: thawing loss = [(W3–W4)/W3] × 100. After the thawing loss was determined, the fillets were cooked in a warm water bath (80°C) for 30 min. Afterward, they were blotted dry and weighed (W5) to record cooking loss. Cooking loss was calculated using the following formula: cooking loss (%) = [(W4-W5)/W4] × 100. Drip loss, thawing loss, and cooking loss were used to evaluate the water-holding capacity.

To assess marinade performances, meat was cut in order to obtain parallel cut samples (8 × 4 × 2 cm, weighing about 80 g), which were individually labeled and marinated by the addition of 20% marinade solution (6% sodium chloride and 1.8%

sodium tripolyphosphate) using a small-scale vacuum tumbler (model MGH-20, Vakona Qualitat, Lienen, Germany). The tumbling time was 40 min under vacuum (-0.95 bar) (two working cycles of 20 min/cycle and one pause cycle of 5 min). After tumbling, samples were weighed again, and the difference in weight was used to determine marinade uptake. Marinade uptake was calculated based on carcass weight before marination (W1) and its weight after marination (W2), according to the following equation: marinade uptake (%) = [(W2 –W1)/W1] × 100

Statistical analysis **:** Statistical analysis was performed in R for Windows (version 3.5.1). All data were checked for outliers and the normality of the residuals. Slaughter yield and meat quality were analyzed using a general linear model (GLM) with "zinc source" and "vitamin E level" as fixed factors and block as a random factor (factorial analysis). In the two-factorial analyses, when there was no significant interaction or no trend, only the main effects were taken into account. The differences were considered statistically significant at $p < 0.05$ and considered tendency at $0.05 < p < 0.1$.

Results

There were no interactions observed between the dietary zinc source and vitamin E level for total slaughter yield and the different meat quality parameters (Table 2). A tendency (p = 0.052) for live body weight was observed for the zinc source. Broilers that were fed a diet supplemented with ZnAA tended to have a higher slaughter weight than broilers that were fed diets supplemented with $ZnSO_4$.

Table 2. Effect of the supplemental zinc (Zn) source and vitamin E level (Vit E) on live weight and carcass composition of broilers at slaughter age (day 37).

Treatment	Live weight (g)	Breast weight (g)	Carcass yield (%)	Breast (%)	Drumstick (%)	Thigh (%)	Wing (%)
Zn source (Zn)							
1. ZnSO4	2,843	341.9	71.34	32.25	12.83	22.63	9.93
2. ZnAA	2,950	352.5	71.69	33.31	12.60	21.96	9.82
P-value	0.052	0.032	0.346	0.005	0.224	0.494	0.383
Vitamin E dose (E)							
1. 50 IU/kg	2,893	353.8	71.88	32.83	12.65	22.20	9.92
2. 100 IU/kg	2,913	340.7	71.15	32.73	12.79	22.39	9.84
P-value	0.677	0.807	0.098	0.768	0.425	0.750	0.572
Interaction Zn X E							
P-value	0.292	0.309	0.916	0.208	0.823	0.952	0.352
SEM	48.33	14.03	0.48	0.37	0.21	0.95	0.13

ZnAA, zinc amino acid complex; E, vitamin E; SEM, standard error of the mean.

Only a main effect of the zinc source on breast yield and certain meat quality parameters was observed, whereas no main effect of the vitamin E level was observed. The zinc source significantly affected the breast yield and water-holding capacity of the breast meat. A higher breast meat yield was observed for birds that fed a diet supplemented with ZnAA than birds that fed a diet supplemented with $ZnSO_4$. Breast meat of birds that were fed a diet supplemented with ZnAA was characterized by a significantly lower drip loss and thawing loss than breast meat of birds that were fed a diet supplemented with $ZnSO_4$. No effect of dietary treatment on cooking loss was observed. Dietary treatment did not significantly influence marinade uptake in breast meat (Table 3).

Discussion

Broilers reared under high temperatures often show lower meat yield and impaired meat quality [6]. Breast meat of broilers exposed to chronic heat stress results in pale meat color [18,19], decreased WHC [20,21], and increased cook and drip losses [22] and is characterized by an increased denaturation of sarcoplasmic or myofibrillar proteins and a lower WHC [6]. Additionally, higher breast meat yields are often associated with lower meat quality, characterized by a higher drip and cooking loss and lower marinade uptake [3,5].

Interestingly, in the present study, it was observed that supplying zinc as ZnAA, resulted in increased yield in breast meat, characterized by lower drip and thawing losses, as compared to supplying zinc as $ZnSO_4$. The lack of differences in cooking

loss is probably due to the fact that fillets used to assess thawing loss were also used to assess the cooking loss. The increased breast yield might be partly attributed to the tendency for an increased live body weight of broilers supplemented with ZnAA, as modern broilers are selected for increased yield of pectoralis major muscles [24]. A previous study [25] showed that ZnAA can improve performance under thermoneutral conditions. As zinc plays an important role in normal development and growth, it is possible that zinc supplements with increased bioavailability may better support growth under heat stress conditions. Although this could not be concluded from this study, no thermoneutral control group was included. Therefore, further research needs to be performed to confirm this hypothesis.

Table 3. Quality characteristics and functional properties of breast meat of broilers at slaughter age (day 37)

Treatment	pH	L*	a*	b*	Drip loss (%)	Thawing loss (%)	Cooking loss (%)	Marinade uptake (%)
Zn source (Zn)								
1. ZnSO4	6.17	57.95	7.45	15.30	5.44	10.93	20.95	8.9
2. ZnAA	6.14	58.16	8.09	16.03	4.09	8.07	21.18	9.4
P-value	0.968	0.623	0.104	0.300	0.027	0.026	0.401	0.066
Vitamin E dose (E)								
1. 50 IU/kg	6.13	58.54	7.88	16.07	4.59	9.35	20.98	10.0
2. 100 IU/kg	6.18	57.67	7.66	15.26	4.94	9.66	21.15	8.3
P-value	0.685	0.858	0.782	0.353	0.582	0.819	0.765	0.052
Interaction Zn X E								
P-value	0.427	0.864	0.742	0.524	0.818	0.425	0.452	0.924
SEM	0.05	1.01	0.62	0.64	0.05	0.01	0.01	0.37

ZnAA, zinc amino acid complex; E, vitamin E; SEM, standard error of the mean; L*, lightness; a*, redness; b*, yellowness.

Zinc supplementation as such or increasing supplementation levels might decrease drip loss and improve the water-holding capacity of the meat under thermoneutral conditions [26,27]. Trace minerals help to sustain the production in animals, improve nutrient utilization and at the same time effectively neutralize the oxidant stress and enhance the compromised immune system of heat-stressed birds [28]. As the requirements for trace minerals increase during heat stress, the inclusion of a more readily available zinc source, such as ZnAA, might be more efficient in reducing the adverse effects of heat stress on meat quality [13,14,29]. In addition, it's been reported that increased dietary supplementation of Zn can upregulate the expression of Zn containing superoxide dismutase [30]. As the negative impact of high ambient temperatures on meat quality is mainly caused by oxidative damage to the skeletal muscle [6], the improved quality traits when supplying ZnAA could be ascribed to improved support of the antioxidant defense system [30,31]. Indeed, a previous study [25] showed that ZnAA could decrease the activity of the glutathione peroxidase in plasma on day 36, while malondialdehyde levels did not differ, indicating that ZnAA might better support the oxidative status.

Although it has been acknowledged that vitamin E has a positive effect on meat quality by protecting membranes against lipid oxidation, thus reducing drip loss in meat [32,33], no effects could be observed when the dietary vitamin E level was increased in the present study. It is possible that the increase in the level of vitamin E was insufficient to create an impact on meat quality and yield under these conditions; in the recent literature, supplementation at a level of 250 mg/kg was advised to improve meat quality in broiler chickens [34].

Overall, it can be argued that an organic form of Zn, in ZnAA, which is characterized by improved bioavailability [15,25], might be able to better mitigate lipid and protein oxidation in post-rigor breast muscles and increase both the water-holding capacity and water-binding ability. However, as no thermoneutral control group was incorporated in this study, the observed effects of the zinc source cannot be generalized as a solution for the negative effects of heat stress. Therefore, further research needs to be performed to elucidate the underlying mechanism concerning the effects of zinc sources on meat yield and quality.

Conclusion

Comparing ZnSO4 with more readily available ZnAA shows improved breast meat yield and increased water-holding capacity in broilers exposed to chronic cyclic heat stress at the end of the production cycle. Moreover, the beneficial effects of ZnAA on breast meat yield and quality seem to be independent of the vitamin E level and increasing the vitamin E level has no additional beneficial effects.

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IS[15] Emerging Foodborne Diseases of Poultry Meat and Challenges For Sustainability

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Summary

Poultry meat is one of the most affordable animal sources of complete protein but it is increasingly a vehicle for foodborne pathogens. The majority of bacterial foodborne pathogens do not cause significant disease in the bird, making it difficult to identify problem flocks/birds by clinical signs. These pathogens primarily include nontyphoid *Salmonella* and *Campylobacter* which behave as normal flora organisms in growing birds, although Salmonella can cause mortality and diarrhea if hatchlings are exposed. Pathogens such as Listeria and Clostridium perfringens can contaminate poultry meat in processing resulting in human disease if the food is mishandled. Furthermore, emerging pathogens such as avian pathogenic E. coli, which have acquired uropathogenic virulence factors, and E. albertii, which have acquired enterohemorragic virulence factors, have been shown to colonize poultry thereby potentially serving as a vehicle for dissemination to humans. Therefore, significant resources must be applied in order to identify infected flocks or to prevent colonization with these pathogens.

Introduction

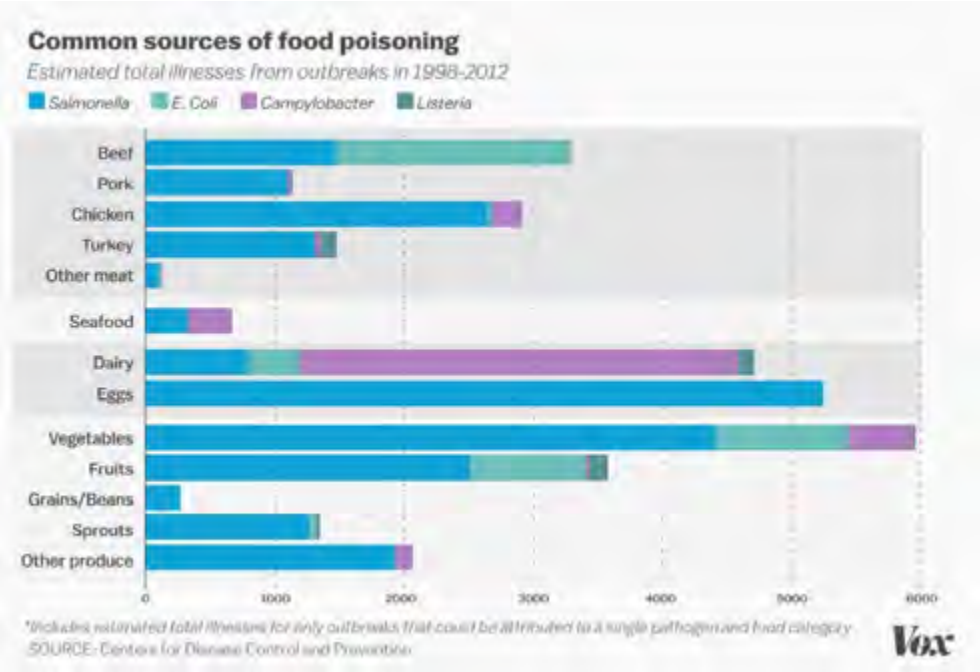
The burden of foodborne illnesses on society. Food safety has become one of the most important public health issues in the world. The WHO's first global estimates of foodborne diseases show that as many as 600 million, almost 1 in 10 people, fall ill every year from eating contaminated food and 420,000 die as a result (1). Diarrheal diseases are responsible for more than half of the total cases, resulting in 550 million illnesses and 230,000 deaths each year, with 220 million cases of diarrhea and 96,000 deaths in children alone. The diarrheal pathogens are associated with eating raw or undercooked meat, eggs, fresh produce and dairy products contaminated by norovirus, *Campylobacter*, nontyphoidal Salmonella and pathogenic E. coli. However other contributors to the global burden of foodborne diseases include typhoid fever (typhoidal Salmonella), hepatitis A, Taenia solium (tapeworm), and aflatoxins. A description of the pathogens can be found at https://www.who.int/en/news-room/fact-sheets/detail/food-safety.

Narrative

Many diseases, such as those caused by non-typhoidal *Salmonella*, are a public health concern across all regions of the world. Other diseases, such as typhoid fever, foodborne cholera, and those caused by pathogenic *E. coli,* are much more common to low-income countries, while *Campylobacter* is an important pathogen in high-income countries. In 2018 the United States Centers for Disease Control and Prevention reported that 9 million of cases of foodborne disease occurred in the US in the past year due to 31 known causes with an additional 38 million cases due to unspecified agents (2). The top five pathogens included Norovirus (5M cases), nontyphoidal *Salmonella* (1M cases), *Clostridium perfringens* (1M cases), *Campylobacter* (850,000 cases) and *Staphylococcus aureus* (250,000 cases) therefore the majority are due to bacterial pathogens. Illnesses leading to hospitalization are generally associated with nontyphoidal *Salmonella* (20,000), Norovirus (15,000), *Campylobacter* (8,500), *Toxoplasma* (4,500), and *E. coli* (2,000) with *Salmonella, Toxoplasma,* and *Listeria* leading the way as causes of deaths. The figure below shows the distribution of disease associated with different foods.

Consumption of poultry and poultry products is a recognized risk factor for foodborne outbreaks of salmonellosis and campylobacteriosis and in fact, most cases of *Salmonella enteritidis* are associated with eggs. In many countries, poultry account for a significant portion of foodborne outbreaks, especially those caused by *Salmonella* and *Campylobacter*. Many governments have invested significant resources in diagnosis and prevention of foodborne disease and to a large degree much of the resources are dedicated to epidemiological investigations to identify the source of outbreaks. These resources have included a disease surveillance system which have identified poultry and poultry products as common sources of *Salmonella* and *Campylobacter* in human foodborne disease. In response, the United States Department of Agriculture implemented the Hazard Analysis of Critical Control Points (HACCP) program in meat processing plants to offer quality control and surveillance in order to reduce the amount of *Salmonella* contamination associated with poultry. And while these efforts have

reduced the portion of *Salmonella* illnesses resulting from poultry, it has not reduced the overall incidence rate of *Salmonella* in the US. In fact, red meat, vegetables, fruits, and spices are now dominant sources of the pathogen in foodborne outbreaks (3).



Emerging foodborne pathogens associated with poultry. While *Salmonella* and *Campylobacter* are recognized as the major poultry-borne pathogens causing illnesses, there are a number of emerging pathogens and issues in the industry. These generally represent new evolving strains or opportunistic pathogens that have colonized birds or contaminated a system within poultry production.

Conclusion

Problems relating to foodborne disease control. By integrating the information gathered from surveillance programs, many countries have been successful in early detection of outbreaks and identifying the foodborne source of cases. However, these epidemiological investigations have not greatly augmented the poultry industry's attempts to reduce the levels of *Salmonella* and *Campylobacter* present within the various levels of the integrated poultry production system. While on-farm sanitation, feed treatments and rodent control can reduce exposure of poultry to foodborne pathogens present in the commercial poultry house environment, some are egg-transmitted from grandparents to parental stock to meat birds. Therefore, control strategies must be multifactorial focusing on breeder flocks, microbiological quality control of feed, hatchery management and farm sanitation. Bird specific treatments include probiotics, yeast, competitive exclusion and vaccination. The commercial poultry industry is unique among the meat producers in developed countries that it is an integrated food production system where the poultry companies own the birds and the processing plants. This enables more effective application of control measures.

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OP41 Elimination of *Staphylococcus aureus* in Chicken Doner and Prevention of Toxin Formation by Bacteriophage Application

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Abstract

In this study, it was aimed to investigate the effect of bacteriophage application on biocontrol and toxin production of *S.aureus* in chicken doner food model. For this purpose, Staf PI-901 phage, which was determined as lytic against *S.aureus*, was applied to chicken doner samples contaminated with low, medium and high (2, 4 and 6 log CFU/g) levels of *S.aureus* D4508 and incubated at 37°C which was determined as the average temperature in commercial doners. The reduction in the number of *S.aureus* was determined by plating at certain hours (0., 30., 60., 90., min. and 3., 6., 24. hours). In the study, the effect of bacteriophage application on staphylococcal enterotoxin formation in low, medium and high level of *S. aureus* in chicken döner kebab was also investigated. The highest reduction in the number of *S.aureus* in chicken doner samples treated with bacteriophage was observed as 3.33 log CFU/g at the 6th hour. Also in the study, unlike the control groups, in all phage-treated groups within the first 6 hours *S. aureus* level was kept below 6 log CFU/g, which is considered the threshold value for toxin formation, According to the results of the study, although staphylococcal enterotoxin formation was observed from the 3rd hour of incubation in K6 (6 log CFU/ml) and from the 6th hour incubation of K2 (2 log CFU/ml) and K4 (4 log CFU/ml) control groups, no toxin was formed up to 6 hours of incubation at any level of contamination in the phage treated groups (F2, F4 and F6). The results of this study revealed that bacteriophage application can be used as an effective biocontrol method in terms of decontamination of *S. aureus* and preventing the toxin formation in chicken doner food model.

Keywords: Decontamination; doner kebab; chicken meat; staphylococcal enterotoxin.

OP42 Antimicrobial Resistance in Broiler Chicken Originated *Klebsiella Pneumoniae* Isolates

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Abstract

Poultry products are sources of enteric pathogens transmission to humans. *K. pneumoniae* leads these agents and is frequently isolated in antimicrobial resistance (AMR) associated nosocomial infections. In this study, it was aimed to investigate AMR in broiler chicken originated *K. pneumoniae* isolates. In the phenotypic AMR test, 14 different antimicrobials were analyzed by Kirby-Bauer disk diffusion method. In the genotypic AMR test, the presence of plasmid-mediated β-lactamase resistance gene bla_{TEM} and colistin resistance genes *mcr1* and *mcr2* were examined by polymerase chain reaction (PCR). As a result of the phenotypic AMR test, 100% resistance to AMP and 100% suspensibility to MEM were detected, respectively from the β-lactam group penicillins and carbapenems. It was determined that the isolates were highly susceptible to β-lactam group cephalosporins (CAZ, 53%; CRO, 97%; CTX, 80% and FOX, 70%). A significantly high amount of multidrug-resistance (MDR) *K. pneumoniae* was found in 63%. The dominant MDR pattern was the AMP-C-S-TE-W-SXT hexa-pattern (26%). As a result of the genotypic AMR test, 70% of the isolates were found to be bla_{TEM} positive, while *mcr1* and *mcr2* positive isolate was not detected. The high bla_{TEM} positivity was associated with this gene is responsible for more than 80% of AMP resistance in enteric pathogens. Consequently, in terms of high AMP resistance, phenotypic and genotypic AMR test findings were found to be compatible.

Keywords: Antimicrobial resistance, β-lactam, broiler, *K. pneumoniae*

OP43 Extended Spectrum Beta-Lactamase (ESBL) Resistance in *Enterobacteriaceae* (Enterobacterales) Isolates Isolated from Chicken Cecum and Neck Skins

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Abstract

Increasing antibiotic resistance in microorganisms is encountered due to excessive and unconscious use of antibiotics in both animals and humans, as well as many factors. This situation poses a serious risk in terms of public health and food safety. Many national and international organizations such as the World Health Organization (WHO) and the European Food Safety Authority (EFSA) point out the importance of this problem and warn national authorities to understand the development and spread of antibiotic resistance. Epidemiological studies carried out in recent years have revealed that the foods we consume play an extremely important role in the spread of resistant bacteria and genetic elements encoding resistance. Beta-lactam group antibiotics are the most preferred antibiotic group today due to their low side effects and their bactericidal nature. These antibiotics have a bactericidal effect by inhibiting bacterial cell wall synthesis. β-lactam antibiotics have found wide use in community diseases, in the treatment of hospital-acquired infections and in veterinary practice. Resistance to β-lactam antibiotics by bacteria has gradually increased due to their widespread preference. Today, the development of multi-antibiotic resistance has been increasingly observed in enteric bacterial strains that produce beta-lactamase, which inactivates extended-spectrum beta-lactam antibiotics. These strains increase the morbidity and mortality rates in the infections they cause, and adversely affect the course of treatment. Therefore, it is of great importance to detect these types of beta-lactamases, which show multiple resistance to antibiotics, in the foods we consume. In this context, in this study, it was aimed to isolate extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* from chicken cecum and neck skin samples taken from poultry slaughterhouses, to determine ESBL production phenotypically and to determine the minimum inhibition concentration (MIC) values of isolates against different antibiotics.

IS[16] Biotechnology; Critical Solution for Sustainable Living, Environment and Food Production

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Abstract

The rapidly increasing world population, reaching 10 billion by 2050, necessitates an increase in agricultural production. In fact, current agricultural production technologies are sufficient to feed all of this increasing world population. However, various political factors are the biggest obstacle to fair distribution. In any case, the productivity obtained per unit area must increase by 50% to feed the population that will continue to increase for the next 30 years. Of course, mitigating global warming to some extent by using agricultural pesticides and chemical fertilizers that are proven to be harmful to the environment more consciously and reducing carbon dioxide and methane gas emissions resulting from agriculture to net zero are essential conditions for sustainable production.

Introduction

The world population, which reached 8 billion last year, is expected to reach 10 billion by 2050. In addition to the increase in the number of people, changes in consumption habits due to the increasing level of welfare, for example, increased demands for the consumption of more animal protein, fresh fruits and vegetables, require an increase in agricultural production. This is possible in two ways: either to increase the yield per unit area on currently cultivated agricultural lands or to convert natural habitats such as forests and pastures into agricultural land. In fact, existing agricultural production technologies, the foundations of which were laid by the "Green Revolution" of the 1960s, are sufficient to feed all of this increasing world population. However, various political factors are the biggest obstacle to fair distribution. In any case, the productivity obtained per unit area must increase by 50% to feed the population that will continue to increase for the next 30 years. Of course, mitigating global warming to some extent by using agricultural pesticides and chemical fertilizers that are proven to be harmful to the environment more consciously - or not using them at all - and reducing carbon dioxide and methane gas emissions from agriculture to net zero are essential conditions for sustainable production. Sustainable concentration is a phenomenon that should be addressed together with concepts such as sustainable development and green economy. We can define sustainable development in its most general form as "Development that meets today's needs without jeopardizing the ability of future generations to meet their own needs." In parallel, it is possible to define sustainable concentration as agricultural production that meets the current food needs of the world population by using all kinds of innovative technologies on existing agricultural areas without harming natural resources and the environment.

There are four main factors that threaten the world we live in and its natural resources, that is, the world's biological carrying capacity. The first of these is the increasing world population and the distribution of this population among countries or regions. 20% of the world's population lives in developed countries, and the remaining 80% lives in developing countries. The tragic part is that the population living in these developed or industrialized countries consumes a significant portion of the world's resources; Likewise, they cause more environmental destruction. Those most affected by this environmental destruction are the 80% poor population. The most talked about environmental destruction lately is undoubtedly natural disasters caused by global climate change.

Here, the primary culprit is the rapid increase in carbon dioxide levels in the atmosphere, especially as a result of greenhouse gas emissions. Unless we limit this rate, which is currently at 420 ppm, to 450 ppm by the end of this century, global warming is expected to exceed 2 degrees. Although the optimistic target is 350 ppm, the current situation shows that it will exceed 450 ppm and reach 550 ppm. Of course, there is also a 60% destruction of the ecosystem, including natural habitats. Efforts to stop this are not very satisfactory. Meanwhile, the uncertainty and surprise results in calculating the consequences of the damage to the ecosystem should not be ignored. In other words, what seems like 1% damage to the environment at first glance can have unexpected consequences in 99% different places.

A very serious contradiction emerges here. NGOs based in EU countries, which oppose GMOs and new plant breeding techniques that are now developing, including the Green Revolution, believe that they will ensure sustainable production with organic agriculture. For this purpose, they prepared a comprehensive program called the Green Deal and made the agricultural supports and rural development projects provided within the framework of the Common Agricultural Policy contingent on environmental impact assessments. This is of course a reasonable approach at first glance. The Green Deal,

which is planned to be supported with a total budget of 1 trillion euros until 2050, aims to harmonize all economic activities that cause global climate change with sustainable development principles. The Farm to Fork and Biodiversity Strategies include reducing the use of chemical fertilizers and pesticides by EU farmers by 20% and 50% respectively, halving the use of antibiotics in livestock, and reducing the use of chemical fertilizers and pesticides by 10% of farm land by 2030.

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OP[44] Biosafety Practices In Broiler Production and Sustainability

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Abstract

Due to factors such as rapid population growth, global warming, and the rapid disappearance of agricultural land, along with advancing technology, poultry meat production has shown unprecedented growth in the last 50 years. The rapid development of integrated facilities and the transition to large-scale industrial production in all parts of the world have made high-yielding and profitable poultry meat production increasingly important every day. The first step in producing quality and economically valuable clean food, free from diseases, is healthy poultry farming, or “Safe Farms”.

The doubling of poultry production worldwide in the last decade shows how high consumer demand is. However, poultry production is also faced with disease outbreaks in response to high supply and demand. Due to factors such as high animal density, high revenue, and industrialization of production, biosecurity measures are becoming even more important.

To achieve the goal of healthy production, biosecurity measures are at the forefront with the elimination of risks, applicable strategies, and uninterrupted communication network.

Key words: Biosecurity, culture, sources of contamination, struggle

OP45 Turkey's Broiler Meat Export Forecast Using by Artificial Neural Networks Method

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Abstract

In the study, Turkey's monthly broiler meat export values were estimated in US Dollars by using MultiLayer Perceptron (MLP) method, which is one of the Artificial Neural Networks (ANN) methods. First of all, the factors affecting the export demand for broiler meat were investigated and determined. The factors affecting the broiler meat export demand were the independent variables. The dependent variable of the established model; is the monthly export value of broiler meat (USD). The independent variables are the export amount of broiler meat (kg), the consumer price of broiler meat (TL), the amount of broiler meat produced (tons), the number of slaughtered broiler chickens (thousand), the broiler chicks produced (thousand), the broiler chicks hatched (thousands), broiler chicken feed price (Ton/TL) and average dollar rate (TL). All data have been compiled on a monthly basis. In the study; Using the monthly data of the previous years (2013 January-2022 April), the chicken meat production values (USD) that Turkey will export in the next 12 months (2022 May-2023 June) are estimated

Keywords: Artificial Neural Networks, Export Forecasting, MultiLayer Perceptron (MLP), Poultry.

IS[17] The Use of Gut Health Biomarkers to Improve Sustainbility of Broiler Chicken Production

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Abstract

When broilers are produced without in-feed and preventive antibiotics, gut health syndromes, diseases and associated performance losses are rather common. While it is easy to diagnose diseases such as coccidiosis and clinical necrotic enteritis, more subtle gut health disorders affecting animal performance are more difficult to diagnose. Poor performance is often associated with changes in gut morphology such as reduced villus length as well as increased inflammatory conditions. This is associated with microbial shifts in the intestinal tract, a condition also referred to as dysbiosis. Veterinarians diagnose this condition by macroscopically evaluating gut wall appearance, which is time-consuming and invasive. Because of the technological developments in meta-omics, (host and bacterial) proteins, (mainly bacterial) metabolites and (bacterial) DNA sequences correlating with gut health can be identified. Host biomarkers for gut health found in faecal material are related to cellular damage, leakage of serum proteins in the intestinal contents and inflammation. These may be (semi-) quantified using ELISA or even dipstick methods on-farm. DNA sequences can be quantified using qPCR methods that are also currently available in carry-on equipment. These tools can be used to determine the gut health status of animals and to predict animal performance. They can be used to make a decision whether or not to apply feed additives or other interventions that promote gut health.

Introduction

One of the most sustainable types of production of animal protein is chicken meat production. Chicken production needs less feed consumption per kilogram of produced meat and uses less land and water for both farming and feed production. The major reason for this is the continuous improvement of animal performance, reflected in an ever decreasing feed conversion (kg feed consumed per kg body weight) and reduced time to achieve market body weight (Zuidhof et al., 2014). Continuous improvements in performance parameters include genetic selection for high-performing chicken lines, technological developments in hatching and housing conditions, and feed optimization and management practices that support (intestinal) health. Among the latter, the use of antimicrobial growth promoters is a practice that has been banned in many countries worldwide but the use of therapeutic antimicrobials in the animal production industries is still high, though decreasing. This has created a situation in which the animal and its microbiota are experiencing a big change, as the animal breeds have been used for more than 50 years almost exclusively in a production system where antimicrobial usage was common practice. Reducing or stopping this practice has resulted in different diseases and syndromes, most of which are of intestinal origin. Indeed, about 60% of therapeutic antibiotic usage in broilers is to control intestinal diseases. The move away from antimicrobials has led to increasing concerns about gut health. Bacterial diseases, enteritis, dysbiosis, and poor digestibility are a consequence resulting in poor growth performance of birds. In fact, all these entities have common denominators in the form of microbial shifts that go hand in hand with epithelial permeability increases, inflammation and thus performance losses, and are often related to nutrient excesses in the intestine or feed-derived issues (poorly digestible nutrients, excess of energy or protein levels). The most important intestinal disease entities and syndromes in broilers, with a performance effect, are briefly described in the next paragraph.

Intestinal Diseases and Syndromes in Broilers

The most severe example of a disease that has emerged in broiler chickens after the ban on growth-promoting antibiotics in animal feed is necrotic enteritis, which imposes a significant economic burden on the poultry industry worldwide (Skinner et al., 2010; Kaldhusdal et al., 2016). This disease is typically caused by nutritional excesses in the gut as well as predisposing epithelial defects caused by mycotoxins and coccidia, and it is occurring in animals with the highest body weight gain, so clearly related to production parameters (Moore, 2016; own unpublished data). The causative agents of necrotic enteritis are

netB-toxin containing *Clostridium perfringens* (type G) strains (Rood et al., 2018). Necrotic enteritis can occur as an acute clinical form which is characterized by a sudden increase in mortality, and as a subclinical form which results in a lower weight at slaughter age. In both cases, macroscopic necrotic lesions are found at the mucosa of the small intestine upon necropsy, and thus the intestinal barrier is compromised and severe mucosal inflammation occurs (Prescott et al., 2016).

A disease syndrome that has clearly emerged in the EU broiler industry simultaneously with the ban of growth promoting antibiotics is the so-called 'dysbacteriosis'. This is a poorly described condition of the gut and may or not be a synonym for conditions such as 'wet litter', 'non-specific bacterial enteritis', 'small intestinal bacterial overgrowth', 'malabsorption', and many more. The common clinical denominator is thinning and ballooning of the small intestine, increased water content of faeces and reduced digestibility of feed with undigested residues visible in the faeces (Teirlynck et al., 2011; Ducatelle et al., 2015). In many cases, this is linked to increased feed conversion, decreased body weight and thus poor performance. Moreover, wet litter leads to various additional disease conditions such as pododermatitis, breast blisters and 'hock burn', which are criteria used to evaluate animal welfare. It is generally believed that 'dysbacteriosis' is a condition in which the interaction between the gut microbiota and the host is impaired, such that the gut health is not optimal. All this is probably influenced by nutrition and it is suggested that the altered composition of the gut microbiota induces changes in the gut wall, including morphological changes (villus length decreases, crypt depth increases, epithelial cell damage, …) and inflammatory reactions (infiltration of immune cells in the wall). The combination of a suboptimal microbiota combined with effects on the gut wall would then most likely interfere with digestive processes, eventually leading to poor performance, and induce enteritis.

As broilers often have gut barrier integrity issues (increased permeability), toxins, feed antigens, but also bacterial products and bacteria can cross this barrier and spread systemically. This also aids locomotory diseases that are a consequence of both the high body weight gain and the pressure this puts on the skeleton of the animal, but also of bacteria that attach to bones at different sites in the body. Indeed, lameness in broiler chickens is a significant animal welfare problem, which is increasingly occurring (up to 1% of all animals). Bacterial chondronecrosis with osteomyelitis (BCO) is a disease characterized by bacterial infection in rapidly growing bones under repeated mechanical stress and typically occurs in tibiae, femora and the thoracic vertebrae (Wideman, 2016). The terminology is often confusing and names such as 'kinky back', spondylitis, spondylolisthesis, femoral head necrosis and others are given to describe similar or the same syndromes. It is assumed that bacteria cross the intestinal barrier, enter the bloodstream and hematogenously spread to osteochondritic clefts or to microfractures at the growth plates. When colonizing the growth plates, the bacteria are rather inaccessible to antibiotics and the host immune system, enabling them to induce necrosis. Bacteria that are found in BCO lesions are commensal intestinal bacteria that have translocated through the intestinal epithelium and have spread systemically. Bacterial genera and species that are isolated from BCO cases are, amongst others, opportunistic bacteria including staphylococci, *Escherichia coli*, and enterococci. These kind of disease entities are thus again originating from high performance and at least partly have an intestinal origin. It has been shown that probiotics can affect BCO, again pointing to the intestine as origin of the bacteria that cause the disease (Wideman et al., 2015).

Measuring Intestinal Health in Broilers

Intestinal health is a term that is not yet clearly defined, despite being a focus of major research efforts in the last decade, both in human and in veterinary medicine. It can be described at different levels. In the past, one has used different indirect systems to measure gut health, such as the water content of faecal material. At macroscopic level, optimal gut health can refer to a condition in which there are no observable changes in gut wall appearance as compared to a normal condition. While this is very clear in conditions in which gross lesions are seen, such as in necrotic enteritis and coccidiosis cases, this is less clear and even invisible in conditions that might cause microscopic alterations that affect performance. A method to score gut wall appearance has been validated previously (Teirlynck et al., 2011) and is used by veterinarians for broiler chickens. In this system, in total, 10 parameters are assessed and scored 0 when absent or 1 when present during visual inspection of the intestinal wall at autopsy after which the animal will receive a total score between 0 and 10. Zero represents a normal gastrointestinal tract and 10 the most severe form of dysbiosis. The parameters are (1) 'ballooning' of the gut; (2) inflammation, redness, of the serosa and/or mucosal side of the gut, cranial to the Meckel diverticulum; (3) macroscopically visible and tangible fragile small intestine cranial to the Meckel diverticulum; (4) loss of turgor in longitudinal cutting of the intestine cranial to the Meckel diverticulum within the 3 seconds after incision; (5) abnormal occurrence of the intestinal content (excess mucus, orange content, gas) cranial to the Meckel diverticulum; (6,7,8,9) are identical to (2,3,4,5) but caudal to the Meckel diverticulum and (10) is presence of undigested particles caudal to the ileocecal junction. A low gut wall appearance score thus indicates good gut health. This system however is rather subjective, as it depends on the person who performs the scoring and it is influenced by specific factors such as diet type (e.g. meal vs pellet with regard to the presence

of undigested feed particles). It has, however, been described that the score is associated with histological parameters under certain conditions, including villus length and infiltration of immune cells in the gut wall (Teirlynck et al., 2011). These parameters are much more objective, quantitative and clearly associated with gut health, as they relate to the epithelial surface (villus length) and thus digestibility, and with inflammation. As such, they are associated with intestinal insults that damage the epithelial lining and thus affect performance. Although histological parameters have value in evaluating gut health, these are mainly of importance under experimental conditions (e.g. when testing interventions) and are more difficult to use in field conditions, given their invasive and time-consuming nature. Optimal gut health could thus also be defined as a condition in which no microscopically visible alterations are seen. Other invasive biomarkers are mainly ones that can be found in blood. Acute phase protein (APP) production in the liver can be the result of intestinal bacteria that cause inflammation in the gut, and thus cytokine production by epithelial and immune cells that is sensed by liver cells that produce APP. It can also be the consequence of translocation of bacteria and their products through the gut wall reaching the liver, so that hepatocytes secrete the APP. APP can be measured in serum, but the production of APPs can be triggered anywhere in the body of the animal and not specifically the gut (Langhorst et al., 2008; Eckersall and Bell, 2010). Other biomarkers that could potentially be found in the serum are of microbial origin. Increases in intestinal permeability in poor gut health conditions would lead to translocation of bacteria, LPS and even metabolites such as D-lactate, that can be found in serum. These markers however do not seem to be very reliable because of multiple reasons, including intrinsic differences in the intestinal concentrations on itself that may cause variability in the serum levels (Ducatelle et al., 2018 for more detailed references).

Non-invasive markers are preferred in the field and ideally they should be based on faecal material as this is easy to collect. In addition, mixed faecal samples can be taken so that the gut health status of the whole flock can be evaluated. These markers can be microbial or host-derived. Microbial markers originate from the observation that gut health problems often are associated with shifts in the microbial composition. This has been described in detail for human inflammatory bowel disease, in which *Enterobacteriaceae* have been associated with inflammation and butyrate producing bacteria from the *Ruminococcaceae* family, such as the genus *Faecalibacterium*, have been shown to be depleted in the faeces of diseased individuals (Machiels et al., 2018; Rivera-Chavez et al., 2017). While changes in microbial composition are clear in the case of severe intestinal inflammation, differences can be much more subtle in intestinal disorders with a much less clear phenotype, such as irritable bowel syndrome in humans. The same is true for chickens, in which rather well-described microbial composition shifts have been described in the gut of animals with necrotic enteritis, but, despite numerous studies, it is not easy to identify OTUs that are correlated with intestinal health and animal performance (Stanley et al., 2016). Our group has conducted a number of studies using intestinal inflammation models to describe 16S rDNA sequences that have a correlation with intestinal health (i.e. villus length, immune cell infiltration in the gut wall, and performance) and some general patterns of beneficial and harmful microbial groups can be extracted from these data. Examples are correlations between the reduced abundance of *Faecalibacterium prauznitzii* and *Butyricicoccus pullicaecorum* and conditions that increase the villus length and decrease the $CD3^+$ T-cell infiltration in the small intestinal wall of broilers under experimental challenge conditions, but many more relevant OTU changes occur. In addition to the use of microbial composition and taxa, one could also use functional genes or metabolites as markers. The most well-known example of a beneficial microbial metabolite is butyrate, and functional genes such as the butyryl-CoA:acetate CoA-transferase can be used to quantify the abundance of butyrate producing bacteria in faecal samples (Onrust et al., 2015; De Maesschalck et al., 2015). A lot of other metabolites are involved in gut health and this is a domain in which much progress can be made. Measurements of epithelial permeability can be done using oral administration of compounds that pass through the epithelial layer when damaged and thus can be measured in serum (e.g. FITC-dextran, lactulose/rhamnose, iohexol) (Ducatelle et al., 2018 for references). This is not applicable to field conditions. Host biomarkers for gut health should ideally be associated with gut function, such as digestibility, cellular damage and inflammation, amongst others. In humans, calprotectin, a neutrophil granule protein, is used to quantify gut inflammation and is very useful to assess the severity of intestinal inflammation (Ayling and Kok, 2018). For poultry, our group recently identified a similar protein biomarker in colonic content of animals from an inflammation model. Other markers were identified and were related to inflammation, serum leakage, epithelial cell and tight junction damage. Currently, these are being brought to a practical field assay using ELISA or dipstick assays. An example is ovotransferrin, a protein that is produced in the liver and thus only reaches the faeces when serum leaks through the epithelial cells. The concentration of ovotransferrin increases with the severity of necrotic enteritis and coccidiosis infections, and has been associated with gut damage in not yet published dysbiosis models (Goossens et al., 2018).

Applications and Perspectives

Easy-to-measure biomarkers in faecal samples are of value for the poultry industry for various reasons. First, they can be used to measure gut health in field conditions and justify interventions to promote gut health. These can be the administration of antimicrobials when animals suffer from a diagnosed bacterial infection but can also be the supplementation of a gut-

health promoting feed additive when the animals are not having symptoms. The latter is often the case, and prediction of poor performance will likely be the most important driver for using gut health biomarker tools. Ideally, diagnostic tests for gut health result in the choice of specific feed additives or changes in feed management, depending on the parameter that is affected. Another important application of gut health biomarkers is efficacy testing of newly developed feed additives by the industry. While faecal biomarker proteins (host proteins) and microbial markers (taxa and metabolic pathway genes) have been identified in experimental models (necrotic enteritis, gut inflammation, and more), field applications are likely not to be straightforward for various reasons. Apart from the technical aspects (dipstick or ELISA development), field conditions are very different in different regions worldwide and it might be complex as a lot of preventive antimicrobials are still used. It will be a challenge to convince poultry producers to use a diagnostic tool to justify use of antimicrobials, as the latter are often a cheap and easy certainty for production performance. Educating the poultry production industry on the risks of antimicrobial usage, antimicrobial resistance, and the need to use a strategy of measuring gut health and using non-antibiotic prevention methods is already occurring but needs further effort.

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OP[46] Importance of Gut Microbiota Analysis in Healthy Poultry Meat Production

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Abstract

Campylobacter is the most common pathogen responsible for foodborne zoonotic diseases in humans. The most common source of infection are broiler meat and milk. One of the factors that influence the occurrence of Campylobacter in broilers are related to the gut microbiota. A field case is described to clarify how, a practical non-invasive microbiota analysis tool (PNMA), provides valuable insights in the interaction between the chickens' gut microbiota and pathogens such as Campylobacter and in the interaction between different interventions targeted to reduce pathogen incidence and the microbiota. Two broiler farms with a history of high Campylobacter jejuni and Enterococcus levels (Positive Class, Baseline) were compared with 2 broiler farms without pathogen risk (Negative Class, Baseline) for their difference in microbiota profiles and to evaluate 2 interventions (Probiotic alone or Probiotic + Prestarter) to reduce Campylobacter jejuni and Enterococcus. Lactobacillus was reduced in the Positive farms which indicated an impaired early microbial development, being a predisposing environment for proteolytic bacteria. The Probiotic induced a clear shift towards Lactobacillus at d7 in the Positive Class compared to the Baseline, resulting in a reduction of C. jejuni at d21. The Probiotic in combination with the Prestarter significantly reduced C. jejuni but also other proteolytic bacteria including E. coli, Citrobacter and E. hirae signals compared to the Probiotic alone in the Positive Class.

Introduction

Zoonoses are infectious diseases transmitted from animals to humans through food or water contamination. According to the World Health Organization (WHO), almost 600 million cases of foodborne zoonoses were reported worldwide in 2015, of which 52% were caused by pathogenic bacteria (World Health Organization, 2015). Campylobacter is the most common pathogen responsible for foodborne zoonotic diseases. C. jejuni is responsible for 80%–90% of the diagnosed cases of Campylobacter infections (Facciola et al, 2017). The most common source of infection to Campylobacter are broiler meat and milk. Important factors that influence the occurrence of Campylobacter in broilers are related to the host gut environment, production chain, or farm practices. Several intervention methods have been developed in recent years, such as the combination of strict biosecurity measures, good manufacturing practice (GMP), hazard analysis and critical control points (HACCP), antibiotic alternatives such as probiotics and phytochemicals. However, the prevalence of this pathogen is still high (Mota-Gutierrez et al, 2022). This paper describes how GALLEONTM, a practical non-invasive microbiota analysis tool (PNMA), can be used to gain insight in the interaction between the chickens' gut microbiota and pathogens such as Campylobacter. At the same time, the tool can be used to evaluate different interventions targeted to reduce pathogen incidence and can even be instrumental in the development of new targeted solutions. The benefits of Galleon are clarified by describing a European field case with increased C. jejuni and Enterococcus incidence in broiler chickens.

Development

The intestinal microbiota plays a crucial role in chicken health and production performance (Pedroso et al, 205; Kogut et al, 2019). The maturation of the microbiota of chickens includes rapid successional changes, developing from a simple, to a more complex and diverse composition based on gradual colonization with microbiota (Oakley et al, 2016; Jurburg et al, 2019). Large scale field trials have identified that good performing broiler flocks have a more stable microbiota at earlier age. Delaying or disrupting this development pattern as caused by high antibiotic use or poor chick quality, for example, result in lower performance and increased pathogens risk. Different interventions such as probiotics, prebiotics, postbiotics, phytogenic compounds and organic acids and formulating diets low in fermentable protein have shown to promote early microbiota maturation in broiler chickens (Gao et al, 2017).

Unraveling the interaction between the environmental and host factors and the chickens' gut microbiota is a complex task: there are more than 109 cells/gram bacteria in the ileum and more than 1011 cells/gram bacteria in the ceca (Sekirov et al, 2010); analysis of the gut microbiota by molecular approaches has identified bacterial populations of over 600 species from more than 100 genera; and the relation between bacteria and between bacteria and factors impacting them may also be non-linear. For that reason, Cargill worked more than 10 years on the development of a practical non-invasive microbiota tool called PNMA to extract actionable insights from such big data. The microbiota of cloaca swab samples is quantified using a microarray chip with previously selected DNA populations (biomarkers) which are then analyzed using statistics and non-

linear AI models. With this combination of technologies PNMA provides poultry producers practical, actionable insights on the gut microbiome health of their flocks to help improve animal health, performance, preharvest food safety, and ultimately return-on-investment.

In this field case PNMA was used to investigate the relationship between broiler farms with high and low incidence of C. jejuni and Enterococcus and their gut microbiota, and following evaluation of 2 interventions to reduce Campylobacter and Enterococcus incidence in high-risk farms. Two broiler (ROSS 308, mixed sex) farms in each category (four in total) were selected based on historical scores defined by the local veterinary service and classified as Positive or Negative based on C. jejuni and Enterococcus risk and followed for 3 different cycles. In the 1st cycle the microbiota profiles of the 2 positive and 2 negative farms were compared by collecting cloaca swabs from 24 broilers from each flock at 7 and 21 days of age according to Cargill's protocol and analyzed in Cargill's lab. In the 2nd cycle the effect of a Probiotic (applied in the hatchery) on microbiota profile of positive and negative flocks of the corresponding farms was evaluated. Cloaca swab samples were again collected from 24 broilers from each flock at 7 and 21 days of age. In the 3rd cycle the effect of combining the Probiotic (in the hatchery) with a Prestarter diet on farm on microbiota profile of the same farms were evaluated. Cloaca swab samples were once more collected from 24 broilers from each flock at 21 days of age.

Laser scanner generated fluorescence readings passed data quality control and were standardized. Relative intensity for each bacteria DNA probe was submitted to ANOVA in a factorial arrangement with fixed effect of sampling round (Baseline, Probiotic or Probiotic+Prestarter), farm pathogen class (Negative vs. Positive), age (7 and 21d) and their interaction. Pairwise comparisons between standardized LS-means were made for each bacterium and differences considered significant by passing a FDR (False Discovery Rate) test with P = 0.05.

In the heat map (Fig 1) farm classes and age were grouped based on similarity on vertical clusters while bacteria are grouped based on similarity horizontally. Treatments clustered first by class, then by age. At 7d, samples of the Negative class had a higher signal of cluster 5 rich in Lactobacillus and lower signal of cluster 1 including more Streptococcus and lower gut fermenters but also more proteolytic bacteria. At 21d, samples of the Positive class showed a higher signal of cluster 3, which included Campylobacter and Streptococcus while the Negative class was associated with cluster 4 which included desirable bacteria such as Lachnospiraceae, Ruminococcus and Faecalibacterium.

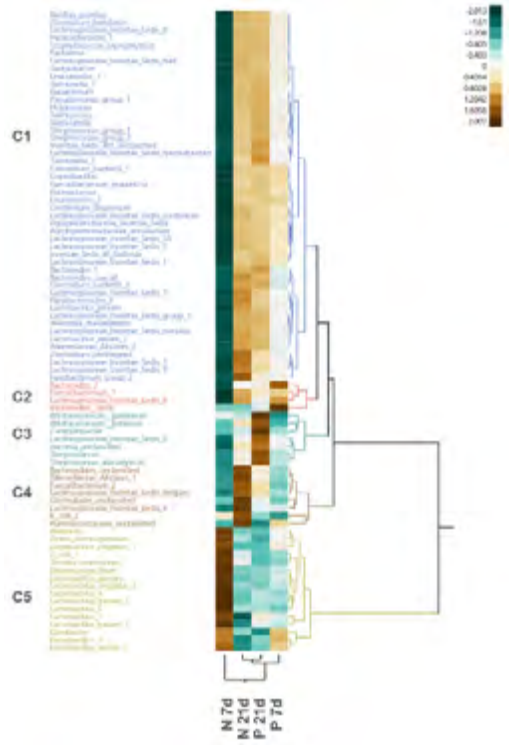

Figure 1. Cycle 1, Microbiota differences between Positive (P) and Negative (N) classes at d7 and d21

At 7d the pathogens Enterococcus and Salmonella were significantly higher for the Positive class in association with higher Streptococcus, although there were no differences for Campylobacter at this age. The Negative class was linked to higher E. hirae, Serratia marcescens and E. coli. At 21d, Campylobacter was higher in the Positive class in contrast with higher Alistipes, Lachnospiraceae, Ruminococcus and Faecalibacterium in the Negative class (Fig 2).

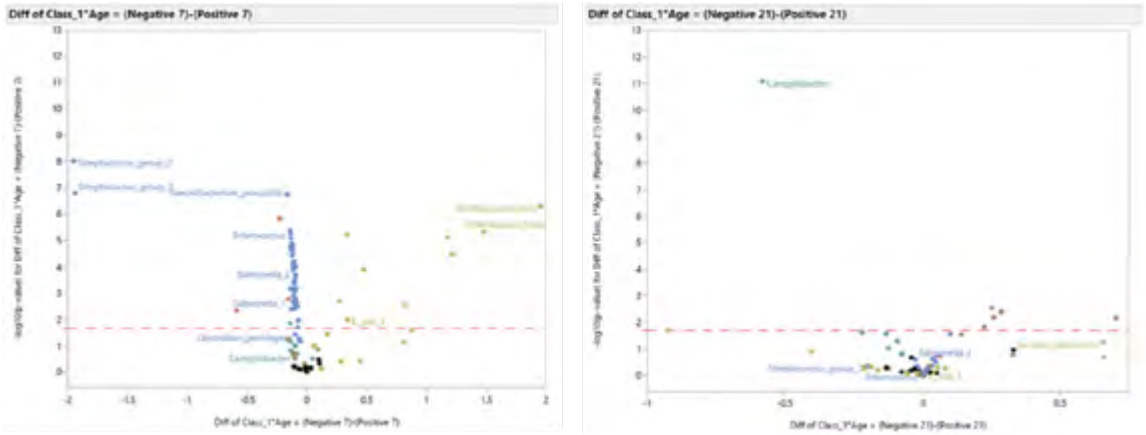

Figure 2. Cycle 1, Volcano plots for the pairwise comparison of microbiota differences between Positive and Negative Classes at d7 and d21

Lactobacillus is a dominating species in normal microbial development as measured by PNMA. Their reduced presence in Positive farms indicated an impaired early microbial development with a less diverse microbiome. This can be a predisposing gut environment to the rise of proteolytic bacteria such as Enterococcus, pathogenic Clostridium and Salmonella. Promoting Lactobacillus in first week instead of Streptococcus in Positive farms could be beneficial by reducing proteolytic bacteria, including Enterococcus and C. jejuni.

Figure 3. shows that at 7d, the Probiotic induced a clear shift towards Lactobacillus in the Positive class compared to the Baseline, which is an indication of a better start. At 21d, the Probiotic induced a shift from high C. jejuni, Streptococcus, Lachnospiraceae and Bifidobacterium in the Positive Baseline towards E. coli, E, hirae, Citrobacter, Faecalibacterium and Alistipes.

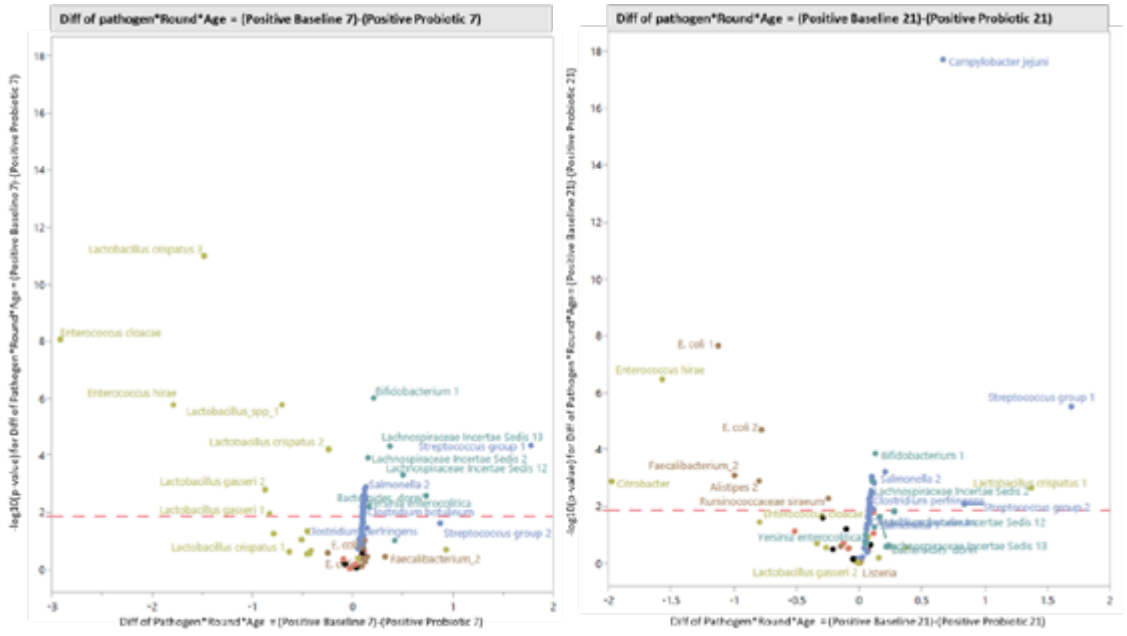

Figure 3. Cycle 2, Volcano plots for the pairwise comparison of microbiota differences between Positive Classes with and without Probiotic at d7 and d21.

Application of the Probiotic in the hatchery significantly improved the composition of the microbiota resulting in a reduction of C. jejuni. However, there was still potential for further improvement and reduction of proteolytic bacteria such as Citrobacter and E. coli.

Figure 4 illustrates that both interventions, the Probiotic alone or in combination with the Prestarter, significantly reduced C. jejuni. The addition of the Prestarter reduced E. coli, Citrobacter and E. hirae signals compared to the Probiotic alone.

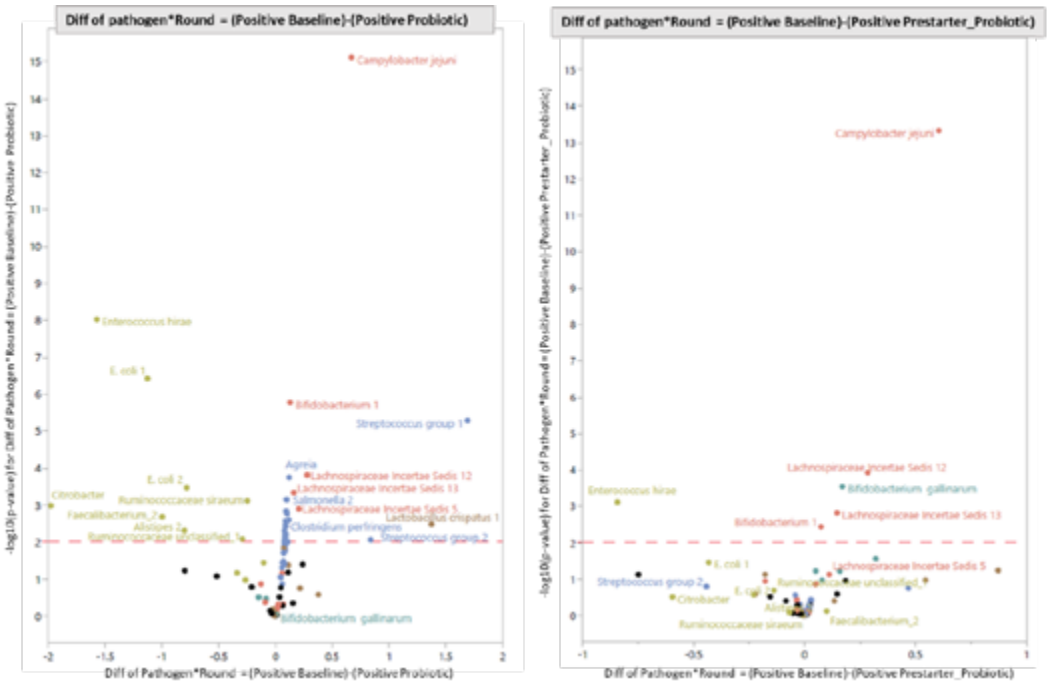

Figure 4. Cycle 2, Volcano plots for the pairwise comparison of microbiota differences between Positive Classes with and without Probiotic (left) and with and without Probiotic + Prestarter (right) at d21

It can be concluded that the Probiotic alone was able to control C. jejuni but when used in combination with a Prestarter it created even a better microbiota profile as seen in good performing birds.

Conclusion

There is a pattern of microbiota development in broilers that is conserved across ages. Delaying or disrupting this development pattern increases pathogens risk as was shown in this field case. It is possible to promote maturation and steer the microbiota towards a more stable and healthy profile by feed formulation and additives such as a Prestarter and Probiotic.

Galleon is a practical non-invasive microbiota analysis tool that allows to study interactions between host and environmental factors and the gut microbiota. The insights generated by Galleon are useful to assess and monitor pathogen risk, to unravel the relation pathogen - gut microbiome, to evaluate interventions to reduce pathogen risk and even to develop new solutions.

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OP47 The Effects of Dietary Monoglyceride Blend Supplementation on Growth Performance, Intestinal Histomorphology and Cecal Short-Chain Fatty Acid Composition of Broiler Chickens

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Abstract

In livestock production, poultry enterprises have a mega share in producing quality protein for human consumption. Such contribution would not be possible if it was not for the genetic improvements in the poultry industry; however, these modifications also brought many challenges along with it. For many years, antibiotic growth promoters (AGPs) assisted the farmers to prevent various infections as well as to cope with performance efficiency. Nevertheless, with the implementation of antibiotic free production, the livestock industry started investing in alternative feed additive strategies mainly targeting the gut health and making it resilient to harmful pathogens. In recent years dietary short and medium chain fatty acids in poultry diets is gaining popularity and various fatty acid products are available in the livestock market solely or in different combinations. In the present study, we investigated the effects of dietary monoglycerides of butyric, caprylic and capric acids on broiler performance, intestinal histomorphology and cecal short chain fatty acid composition. For this purpose, a total of 420, one-day-old male Ross 308, broiler chicks were randomly allocated to five experimental groups using seven replicates each and 12 birds/replicate. Five dietary treatments applied in the study were: (T1, control) non-supplemented diet; treatments T2 and T3 were supplemented (all phases) with monoglyceride blend at doses of 300 mg and 500 mg/kg diet, respectively; treatment T4 was supplemental with 300 mg, 500 mg and 500 mg/kg with monoglyceride blend during starter, grower, and finisher periods, respectively; and treatment T5 was supplemented with 3000 mg, 1500 mg and 0 mg/kg monoglyceride blend during starter, grower, and finisher periods, respectively. The starter, grower, and finisher diets were based on the maize-soybean meal and were offered to birds from 0-14, 14-28, and 28-42 days of age, respectively. All diets were formulated to meet or exceed Aviagen (2019) nutrient recommendations. Water and diets were provided ad libitum throughout the experimental period. All chicks were weighed individually. Body weight gain (BWG) and feed intake (FI) were recorded, and feed conversion ratio (FCR) was subsequently calculated to evaluate growth performance. On d 7, two birds from each replicate were selected for measuring weight and size of gastrointestinal tract. On d 21, two birds from each replicate were selected for ileum histomorphological analysis and short chain fatty acid determination. The birds euthanized on d 42 were also evaluated for carcass traits including carcass yield and relative organ weights (2 birds/replicate). All the data were subjected to ANOVA procedure of the SPSS software, version 14.01 (SPSS Inc., Chicago, IL). One-way ANOVA was used to determine the effects of additive supplementation and significant means were separated using Tukey test. Statistical differences were considered significant at $P \leq 0.05$. Dietary monoglyceride blend supplementation significantly increased broiler BW on d 7 (T2, T3, T4, and T5) and d 28 (only T2 and T4). The results on FCR (d 0-28) showed better results in T4 and T5 groups when compared to control, even though no significant effect was observed when compared to T2 and T3 groups. Cumulatively, no differences were observed in BWG, FI, and FCR among the dietary treatment groups during the overall experimental period. Also, no significant differences were found between dietary treatments for GIT weight/size and all the carcass traits observed on d 7 and d 42, respectively. Furthermore, histomorphological analysis showed significant increase in villus height in T5 groups when compared to control group on d 21. Supplementation of monoglyceride blend to broilers' diets did not influence the cecal concentrations of SCFAs either individually or in total expect an increase in the valeric acid level in T4 group compared to T2 and T3 groups. In conclusion, the results of the present study revealed that usage of monoglyceride blend in broiler diets might be useful in improving performance as well as in modulating intestinal architecture. For future research, challenging environment should be considered which might show more pronounced impact of supplemental monoglycerides at these dosage rates than observed in this experiment.

Keywords: Broiler, intestine histomorphology, monoglyceride blend, performance, short-chain fatty acids

OP[48] Making The Connection Between Improved Gut Health and Meat Quality in Broilers – A Comparison of Field And Research Data

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Abstract

Besides growth performance, slaughter characteristics and meat quality are important parameters determining economical sustainability of broiler production. So far, no clear direct link has been made between gastro-intestinal functionality and meat quality, however several studies indicate an interaction. Muramidase is an enzyme that breaks down dead bacterial cell debris (peptidoglycans; PGNs) in the intestine. These PGNs of dead bacteria cause an (unnecessary) immune response. There are several examples in which muramidase shows to support gastro-intestinal functionality, improves gut health and thereby broiler growth and slaughter performance.

In a meta-analysis including over 80 broiler trials worldwide comparing a control diet with a diet containing muramidase, an improvement of 1.5% ($P<0.05$) in body weight gain (BWG) and 2.5% ($P<0.05$) in feed conversion ratio (FCR) was shown. Different authors have described that by adding muramidase to the diet, nutrient digestibility and absorption are improved (Goodarzi et al., 2019; Sais et al., 2019). E.g., Sais et al., 2019 measured ileal digestibility when comparing diets with and without 35.000 LSU/kg muramidase. Total fatty acid digestibility was improved by 2% ($P<0.05$) on day 9 of broiler age, and on day 35, energy digestibility was improved by 5% ($P=0.0001$) and crude protein digestibility by 3% ($P=0.095$). In this trial, plasma vitamin A levels were increased by 18% ($P<0.05$) on day 9 when muramidase was included in the diet. Vitamin A is an important antioxidant acting through radical scavenging. It reduces inflammation and protects muscle from damaging free radicals.

This might explain the connection to improved carcass quality. When gut functionality is optimal, the partitioning of the nutrients to the carcass and muscles could be optimal as well. The improved plasma vit. A level can be viewed as a marker and suggests other nutrients are better absorbed as well, this can lead to improved oxidative stability (oxidative stress is decreased by carotenoids, vit. E and vit. C absorption), and meat quality (Cavani et al., 2009). Ni et al., 2022 investigated two goose genetics and linked higher breast muscle index to higher ileum villi height/crypt depth ratio and higher SCFA concentrations in cecum (fermentation rate), making the connection between carcass quality and gut health.

The positive effect of muramidase on carcass yield has been shown in field studies (no statistics are available). In one of these studies with 42.000 mixed sex Ross 308 broilers, a control diet with or without supplementation of 35.000 LSU/kg muramidase was compared and growth and slaughter performance were measured. Body weight was improved by 3% and FCR was improved by 2.5%. Addition of muramidase improved carcass weight by 3.5%; breast weight by 5.5%; fillet weight by 11% and thigh weight by 4.5%, while these improvements reduced waste weight by 82% (broken bones, damaged meat etc.).

The data above shows the positive effect of muramidase to broiler performance, nutrient digestibility and absorption and carcass quality. The mode of action of muramidase, balancing the immune system, can be linked to gut functionality, health, and performance. The link with improved carcass quality needs to be investigated further.

IS[18] Histomonosis - Current Challenges in Control of Blackhead Disease in Turkeys

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Abstract

Histomonas meleagridis is a flagellated parasite of poultry. The disease named blackhead disease or histomonosis causes typhlohepatitis in different species of gallinaceous birds, but turkeys are most susceptible. Highly effective chemotherapeutics against histomonosis that have previously been used cannot be administered in poultry flocks to avoid potentially harmful residues in meat. Since the ban of prophylactic and therapeutic drugs outbreaks of the disease increased in turkey flocks.

H. meleagridis infects the host directly or uses the vector *Heterakis gallinarum*. Turkeys that were infected with histomonads via oral or cloacal administration suffered from total mortality in recently performed experiments. Pathological changes are evident in the caecum and liver of birds that show severe inflammation and necrosis. Diagnostic tools include conventional histopathology but specific detection of the DNA of the parasite by *in situ* hybridization or PCR allows differentiation to other protozoa.

Because of the unavailability of prophylactic and therapeutic drugs, new strategies against histomonosis have been investigated in the last years. Chemicals and phytogenic compounds were used to identify an impact against the parasite *in vitro* as well as in infection trials. The effect of some plant derived substances was promising in cultivated parasites but the prevention of the disease in turkeys was limited or not possible at all. However, an effort could be achieved by experimental vaccination of turkeys using *in vitro* attenuated histomonads. Turkeys showed full protection against the challenge during clinical and pathological examination after vaccination. In continuative studies mechanisms of the immunity have been investigated and revealed the involvement of interferon gamma positive cells and different populations of leukocytes like B cells, T cells and monocytes/macrophages after vaccination and challenge.

Histomonosis can severely impair the health of turkeys and no effective options for treatment are available. Recent studies on compounds against the parasite did not show substantial effects against the parasite. In contrast, experimental vaccination of turkeys but also chickens using an attenuated clonal strain of *H. meleagridis* could prevent histomonosis arguing for further research on the developed vaccine candidate.

Introduction

Histomonosis (syn.: blackhead disease) was firstly described by Cushman (1893) in turkeys. Nitrofurans and nitroimidazoles, the only available drugs against histomonosis in Europe, were withdrawn by the authorities due to concerns on product safety (CEC 1995, 2002). Likewise, chemotherapeutics for the use against histomonosis were removed from the market in other countries. As a result, outbreaks of the disease in poultry flocks increased due to the lack of prophylactic and therapeutic options (Hess *et al.* 2015).

H. meleagridis is unicellular, round to ameboid and it is composed of organelles typical for trichomonads (Hess und McDougald 2020). There parasite can be flagellated in the caecal lumen of birds or without flagella after it penetrates tissue. The lumen form propagates in the caecum and can be isolated *in vitro*. Formation of pseudopodia can be observed in microscopical preparations. In tissues the parasite can be found between host cells that can undergo necrosis. Beside the described stages histomonads can express an outer membrane which is characteristic for the resistant phase. Extensive investigations by light and transmission electron microscopy identified cyst-like stages in a clonal culture of histomonads (Zaragatzki *et al.* 2010).

The infection of turkeys and chickens with *H. meleagridis* can occur by direct intake or via embryonated eggs of *Heterakis gallinarum* that harbour the flagellate. In infection experiments it was observed that the direct infection with *in vitro* cultivated histomonads via the oral or cloacal route caused fatal histomonosis in turkeys (Liebhart und Hess 2009). Typical clinical signs of turkeys suffering from histomonosis are ruffled feathers, drooping wings, apathy and sulphur coloured diarrhoea. The mortality of turkeys can be up to 100% as being reported from several experimental studies (Hauck und Hafez 2013). In chickens the disease causes generally milder clinical signs and a lower mortality, but losses of the disease were described to resemble that of coccidiosis (McDougald 2005).

The infection starts with the colonization of the parasite in the caecum leading to severe inflammation and necrosis (Figure 1). The intestinal barrier can be severely destroyed, and the parasite is able to infiltrate blood vessels and reaches the liver via portal veins. As a result, severe inflammation and necrosis occur in the liver. The disease can become systemic when the parasite infiltrates different organs of the turkeys (Grabensteiner *et al.* 2006). Pathological changes caused by *H. meleagridis* are noticed as inflammation of the

caecal wall, bleedings in the mucosa and fibrinous content in the caecal lumen. Liver lesions resemble multifocal areas of necrosis and inflammation that are found as round areas in variable size before they merge in the final stage of the disease (Figure 1). The lesions can be observed in all poultry species that are susceptible to histomonosis with variations in the severity of pathological changes. Necrosis and inflammation can be confirmed by histopathology. For a specific detection and visualization in organs, an *in situ* hybridization or an immunohistochemistry can be applied to distinguish *H. meleagridis* from other protozoa like *Tetratrichomonas gallinarum* (Liebhart *et al.* 2006; Singh *et al.* 2008).

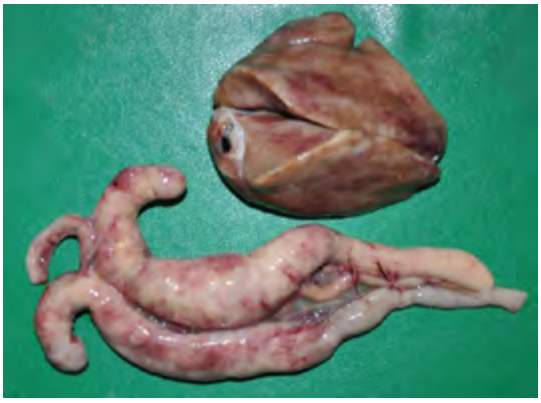

Figure 1. The caeca and the liver of a turkey infected with *H. meleagridis*. The organs show severe inflammation and necrosis.

The need to combat histomonosis is highly evident but there are certain challenges to identify new options for prophylaxis or therapy. In recent years, different approaches against histomonosis were investigated, including the application of chemical or plant-derived compounds and vaccination.

Chemotherapeutics involved the aminoglycosid antibiotic paromomycin which was already found to reduce the mortality of infected turkeys (Lindquist 1962). In a more recent study, the prophylactic effect in turkeys could be confirmed but the antibiotic could not prevent the disease in already infected turkeys (Bleyen *et al.* 2009a). However, it was demonstrated that the use of paromomycin causes significantly higher frequency of antibiotic resistance of intestinal bacteria (Kempf *et al.* 2013). In another work, Nifurtimox, a nitrofuran, was investigated for its effect against histomonosis when applied as feed additive (Hauck *et al.* 2010) but this group of chemicals are not licensed for the use in poultry in Europe, USA and other countries to protect consumers against potential negative effects. Other compounds like boric acid, sodium chlorate or sodium nitrate resulted in no effective prophylaxis against a challenge in turkeys (Barros *et al.* 2020; Beer *et al.* 2020).

Compounds from oregano, cinnamon, thyme, lemon, garlic, rosemary, and other plants were tested for their effect against *H. meleagridis*. *In vitro* experiments using these compounds showed to inhibit the growth of cultured histomonads (Grabensteiner *et al.* 2008; van der Heijden und Landman 2008a; Zenner *et al.* 2003). However, in most of the studies no effect was observed when applied to prevent histomonosis in turkeys (Grabensteiner *et al.* 2008; van der Heijden und Landman 2008b) except a reduction of mortality (Hafez und Hauck 2006) (Table 1). In another work, artemisinin, a compound known for its activity against human malaria was found to have an effect *in vitro* but the prevention of histomonosis turkeys as well as in chickens was not possible (Thøfner *et al.* 2012).

Table 1. Experimental investigations on the effect of plant derived compounds in turkeys infected with *H. meleagridis*.

Botanical / herbal product	***in vivo* effect**	**supplementation**	**reference**
Protophyt® **oils from cinnamon, garlic, rosemary, lemon**	30% reduction of mortality	0.2% via feed and 0.3% via drinking water	Hafez und Hauck 2006
Thymus vulgaris ethanolic extract	100% mortality	1% via drinking water	Grabensteiner *et al.* 2008
Serenoa repens ethanolic extract	100% mortality	1% via drinking water	
Vitis vinifera ethanolic extract	91.7% mortality	1% via drinking water	
*Cucurbita pepe*ethanolic extract	100% mortality	1% via drinking water	
Enteroguard™ lyophilized garlic and cinnamon infusion with active compounds allicin and cinnemaldehyde and other thiosulfonates	100% mortality	500 ppm	van der Heijden and Landman 2008b
Protophyt SP™ oils from cinnamon, garlic, rosemary, lemon	94-100% mortality	3000 ppm via feed	
Protophyt B™ oils from cinnamon, garlic, rosemary, lemon	100% mortality	0.2 % via drinking water	

Artemisia annua-derived materials (i.e. dichloromethane extracts of leaves and pure artemisinin	85-100% mortality;	0.1% via drinking water or 100 ppm via feed	Thøfner *et al.* 2012

In recent years, research on vaccination of poultry was performed due to the demand to control histomonosis. In a challenge experiment, it could be demonstrated that turkeys can be protected from fatal histomonosis by vaccination using clonal *in vitro* attenuated histomonads (Hess *et al.* 2008). In further investigations, the safety and stability of attenuation following consecutive *in vivo* passages could be shown (Sulejmanovic *et al.* 2013). More recently, cross-protection against isolates from different European countries of *H. meleagridis* was demonstrated (Sulejmanovic *et al.* 2016) as well as the protection against a different genotype (Hatfaludi *et al.* 2022). Other vaccination experiments showed protection of turkeys after infection with virulent histomonads and chemotherapy (Bleyen *et al.* 2009b) as well as the application of intracloacally passaged low-virulent histomonads (Nguyen Pham *et al.* 2013).

The interaction of the parasite with the host immune system was the aimed to be investigated in subsequent studies. It was shown that chickens raised an earlier immune response than turkeys in the caeca based on cytokine expression profiles (Powell *et al.* 2009). Infection with *Eimeria* spp., another protozoan parasite, results in the activation of T cells, a cell population that is essential for immune protection against a challenge (Smith *et al.* 2014). The immune response during coccidiosis and histomonosis has certain similarities, which are the severe inflammation, the destruction of intestinal tissue and the expression of cytokines (Powell *et al.* 2009; Rose 1996). The immune system can respond by type 1 or type 2 pathway, depending on the causative pathogen. This was also confirmed in birds, as reported by Degen *et al.* (2005) who demonstrated Th1/Th2 polarization by intracellular and extracellular infection in chickens. The change of immune cells following infection and vaccination was shown by *in situ* hybridization of interferon gamma producing cells in turkeys that responded with an increased number of these cells in the caeca to similar levels that were observed in naïve chickens that are known to be less affected by histomonosis (Kidane *et al.* 2018). This result could be further elaborated by flow cytometry analyses showing that vaccinated turkeys have more interferon gamma producing cells consisting of all major T-cell subsets in the spleen and the liver compared to vaccinated chickens (Lagler *et al.* 2021). Furthermore, vaccination of turkeys with attenuated histomonads led to a reduced cellular response compared to an infection with virulent *H. meleagridis*, resulting in protection (Mitra *et al.* 2017). Most recently, the importance of the innate immune system of poultry was reported by investigating the expression of Toll-like receptors after vaccination with attenuated histomonads (Mitra *et al.* 2021).

Discussion

Histomonosis in poultry and especially in turkeys causes worldwide losses in flocks because of the lack of adequate measurements to prevent birds from clinical signs and fatality (Liebhart und Hess 2020). In the last years, different prophylactic approaches against histomonosis were investigated.

Chemicals like nifurtimox or paromomycin were shown to be effective when applied before the infection (Hauck *et al.* 2010; Bleyen *et al.* 2009a). However, the application of the nitrofuran nifurtimox and the prophylactic administration of antibiotics like paromomycin is not approved in many countries. Other recently investigated chemical compounds did not show an effect against histomonosis of turkeys as recently summarized (Beer *et al.* 2022).

Different plant-derived compounds were examined for an anti-histomonal effect *in vitro* and *in vivo* as recently reviewed (Liebhart *et al.* 2017). Many of the substances showed an effect *in vitro* against the growth of *H. meleagridis*, however, experimental infection of turkeys did not reveal a substantial prophylactic impact. These results highlight the importance of *in vivo* trials to determine the effect in host-pathogen interaction under physiological conditions.

In contrast to the application of chemical or plant derived compounds, vaccination of turkeys showed promising effect to prevent histomonosis (Hess *et al.* 2008). This use of clonal *in vitro* attenuated histomonads as vaccine candidate was found to be safe regarding the clinical outcome and organ lesions (Liebhart *et al.* 2011). Furthermore, protection was established against various isolates and a different genotype (Sulejmanovic *et al.* 2016; Hatfaludi *et al.* 2022). Most important, the loss of virulence was shown to be irreversible after five consecutive passages in turkeys and chickens (Sulejmanovic *et al.* 2013). The mentioned studies were fundamental to assure a safe and effective vaccine candidate to be prospectively applied in poultry flocks.

Vaccination was shown to protect turkeys and chickens from histomonosis but the immune response against the disease was previously not further elucidated. It was known that transferred antibodies do not protect turkeys from the disease (Clarkson 1963) and that the immune system of chickens responds earlier than that of turkeys, elucidated by cytokine expression (Powell *et al.* 2009). In later on performed studies on the immune response, attenuated histomonads were included to compare the effect of the vaccine candidate on poultry as summarized by Mitra *et al.* (2018). It was found that vaccination causes a limitation of pronounced changes of various lymphocyte populations in challenged turkeys (Mitra *et al.* 2017). Furthermore, interferon gamma mRNA positive cells were increased after vaccination indicating a protective ability of these cells (Kidane *et al.* 2018). In a more recent study, histomonad-specific leukocytes were identified systemically in turkeys after vaccination (Lagler *et al.* 2021). Overall, innate, and specifically adapted immune cells of vaccinated turkeys are mandatory to protect birds from a severe challenge.

Conclusion

Histomonosis is a re-emerging disease in countries without admission to administer effective chemotherapeutics, therefore new strategies to prevent the disease need to be applied. Recent experimental investigations demonstrated that application of chemical and plant-derived compounds did not result in an adequate prevention of clinical signs and losses. In contrast, vaccination using *in vitro* attenuated *H. meleagridis* effectively protects turkeys and chickens from histomonosis and is safe in use, arguing for the most promising approach to prevent histomonosis in poultry. Based on recently performed studies it can be concluded that the vaccine primes the immune system of turkeys by changes of different populations of mononuclear cells.

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OP49 Molecular Diagnosis of Hemorrhagic Enteritis Virus (HEV) in Turkeys

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Abstract

Hemorrhagic enteritis is a viral infection that affects turkeys older than 4 weeks of age. Molecular diagnosis of hemorrhagic enteritis virus (HEV) in turkeys was aimed in this study. In the study, spleen samples taken from at least 5 animals showing clinical signs from different turkey flocks were used. DNA extraction from the samples was performed robotically. PCR was performed using primers targeting the hexon gene. Five of the positive samples were sequenced using the Sanger sequencing method. Fourteen (51.8%) of 27 turkey flocks analysed were found to be positive for HEV. All of the samples sequenced with the Sanger sequencing method were found to be compatible with the TAdV-3 reference. The findings showed that HEV is quite common in turkey flocks and it is important to use molecular tests along with clinical findings in the control of the disease.

Keywords: Adenovirus, hemorrhagic enteritis, PCR, turkey

OP[50] Antimicrobial Effect of Commercial *L. Rhamnosus* Cultures on *Van*M-Resistant Enterococci in a Chicken Fillet Model

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Abstract

Vancomycin-resistant enterococci (VRE) are recognized as significant public health pathogens due to limited treatment options. It is very important for public health to prevent the transmission of vancomycin resistance by *vanM*-resistant enterococci to other pathogens and the spread of this resistance gene to the environment. Studies on the antimicrobial effect of LAB cultures on chicken meat are generally limited to *Salmonella*, *Escherichia coli*, and *L. monocytogenes*, and studies on inhibiting VRE are insufficient. In this context, the suppressive effect of commercially produced *L. rhamnosus* cultures on *vanM*-resistant enterococci was investigated in this study. For this purpose, raw chicken fillet samples were contaminated with 4-6 log cfu/ml VRE, then dipped in a solution containing 9 log cfu/ml *L. rhamnosus* and then analyzed, and a decrease of 1.36 and 0.54 log cfu/ml in enterococci counts determined, respectively. It can be observed that the enterococci counts remained below the initial contamination at the end of the 3rd day following the application to the samples with both contaminations. When the results of the study are evaluated, it is concluded that the commercially used *L. rhamnosus* cultures can be used to suppress VRE in poultry meat and products.

SSP01 Optimize Your Phytase Strategy with Axtra® PHY Gold

Bart Hillen

IFF-Danisco

Since the first fungal phytases were developed, significant advancements have been made in this field. After the initial fungal phytases, E. coli phytases largely took their place. In the next stage, phytases derived from other bacteria such as Buttiauxella and Citrobacter, in addition to E. coli phytases, found their place in the market. The new generation of phytases, however, was developed by combining useful parts of numerous bacteria through genetic engineering, resulting in much more effective products. IFF-DANISCO has always been one of the pioneering companies in this field. They develop cost-reducing products, strategies that promote healthy nutrition and increase animal vitality, as well as environmentally friendly products. In 2017, during the 4th Poultry Congress, at the meeting where we announced our official distributorship of Danisco in Turkey as Nutriline, we introduced our new generation product, Axtra® PHY. In the past five years, DANISCO has developed a new product called Axtra® PHY GOLD. We are pleased to introduce our Axtra® PHY GOLD product to you at the 6th Poultry Congress.

Phytate is the main source of phosphorus in plants but cannot be digested by monogastric animals and has strong anti-nutritional effects. In the upper digestive system, at low pH, it forms undigestible complex structures by binding to proteins and amino acids. In the lower digestive system, where pH levels are higher, it reduces the utilization of calcium and trace minerals by binding to them.

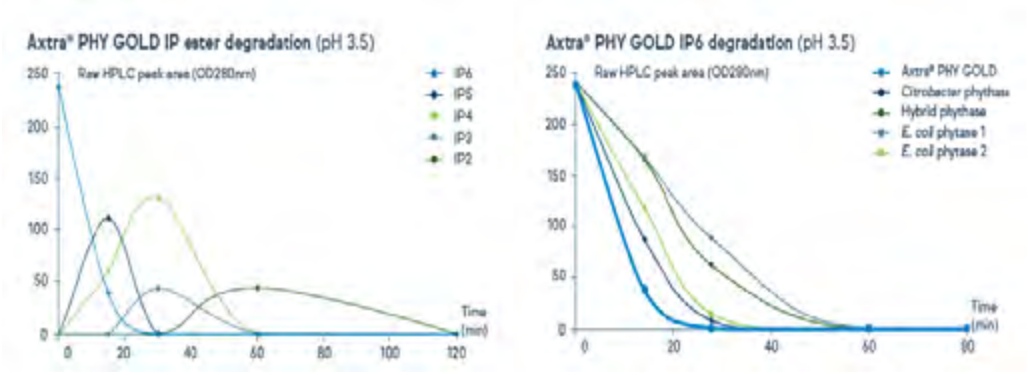
Calcium is necessary for bone development and plays a vital role in various metabolic pathways. However, if ration levels are incorrectly applied, it can pose a significant threat to animal growth.

Due to its low cost, it is generally used in formulations with high safety margins. While this application helps to avoid calcium deficiency, it also means that you can end up with higher calcium levels than expected.

When left uncontrolled, high calcium concentrations with high solubility can seriously affect productivity. This is because increased calcium-phytate binding in the upper parts of the intestine leads to a potential decrease in protein digestibility.

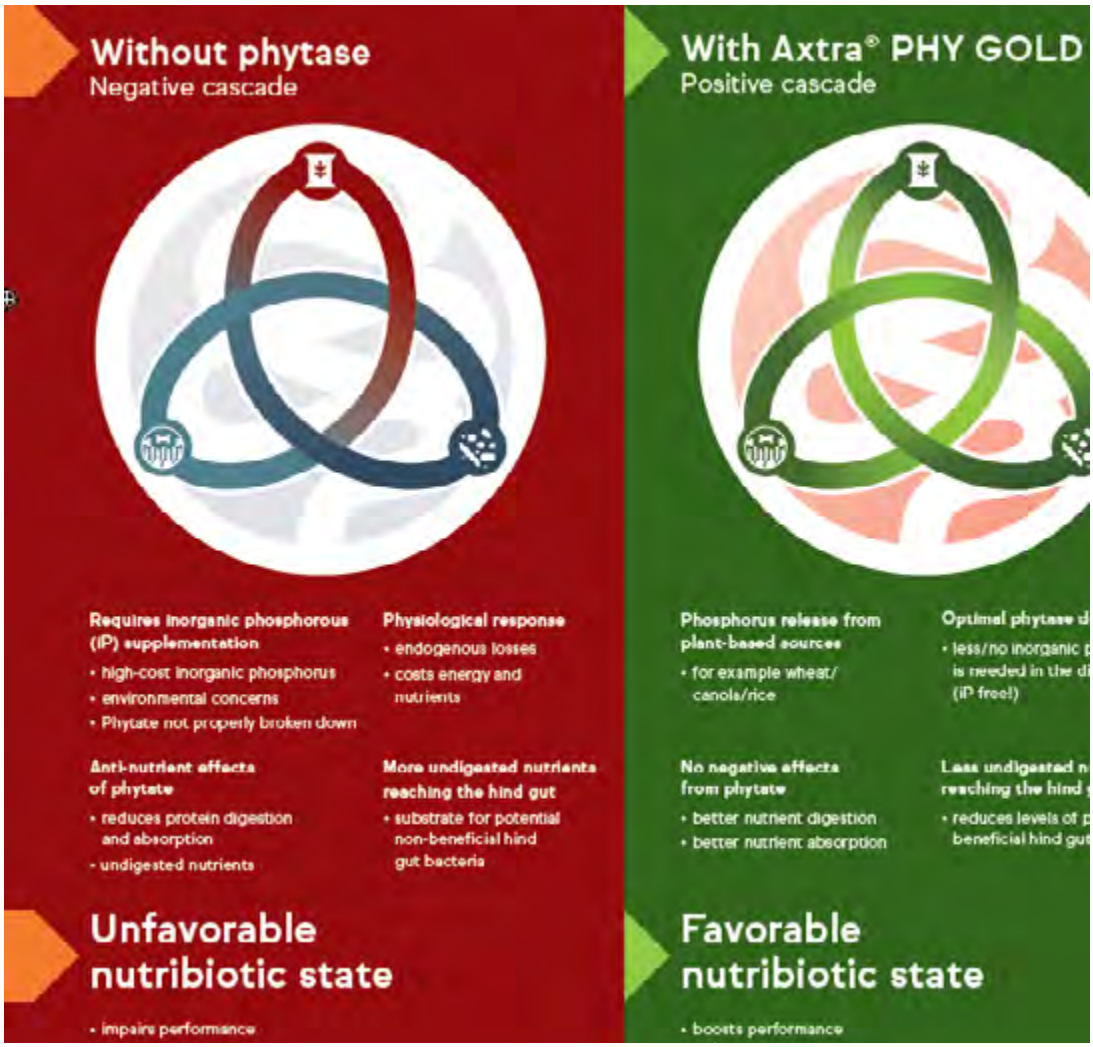
Producers often focus only on the negative effects of low calcium levels. However, studies have shown that high calcium levels are equally problematic.

To neutralize the negative effects of phytate, it needs to be broken down as quickly as possible, which requires a phytase enzyme that works rapidly in the upper part of the digestive system at low pH.



In conclusion, phytate reduces digestibility, decreases energy used for growth, and decreases bone mineralization by affecting the Ca-P balance. All of these negatively impact the animal's nutritional status.

Axtra® PHY GOLD is developed with this idea in mind. Thanks to its superior pH profile, Axtra® PHY GOLD works twice as fast as competing phytases in the market and provides superior bioefficiency.



The applications of phytases today are significantly different from five years ago. Fixed dosage recommendations are a thing of the past. Instead, we can provide you with personalized optimum dosage recommendations that maximize your field performance and economic impact every time.

Axtra® PHY GOLD is supported by matrix values based on phytate and calcium levels within the recommended range of 250-4,000 FTU/kg feed. This allows you to benefit from the highest reliability and flexibility tailored to your working conditions.

Phytases are one of the most valuable feed additives used in the poultry feed industry. Depending on the ration structure, they provide cost savings of around 5-6%. With Axtra® PHY GOLD, you will require minimal or no inorganic phosphorus sources such as DCP-MCP. This not only reduces environmental pollution but also contributes significantly to the industry's economy.

Furthermore, in addition to Axtra® PHY GOLD, when you use our other complementary enzymes, we apply the same data-driven approach to optimize dosage levels.

Over the next few years, phytase will be one of the main focus areas of our R&D, aiming to direct the nutrition science towards ration formulations that do not contain inorganic phosphorus. As a stakeholder in the industry, we are working to meet the increasing societal demand with more sustainable practices. Therefore, our goal is to facilitate the widespread adoption of inorganic phosphorus-free formulations in poultry by further reducing phosphorus usage and making target-oriented adjustments in feeding practices.

We expect Axtra® PHY GOLD to complete its licensing process in May. We hope to introduce it to the Turkish market in the second half of the year. It has been used in the United States and some countries outside the EU for almost two years.

SSP02 Using Enzymes and Direct-Fed Microbials to Facilitate Optimal Intestinal Conditions for Digestion and Absorption

Dawn Reinhoudt

IFF-Danisco

High production costs and sky-rocketing prices for (imported) raw materials emphasize the need for efficient broiler meat production. The extent to which broilers can efficiently convert feed into animal protein is determined by various factors including genetics, chick quality, housing conditions and biosecurity, flock management, climate, feed composition and quality and bird health.

From a nutritional perspective, ingredients that are difficult to digest can result in undigestible fractions which negatively impact feed conversion rate increasing feed costs. To increase digestibility of nutrients, it has become common practice to include exogenous enzymes in broiler diets.

From an animal health perspective, even if feed digestibility is optimal, impaired gut function or damaged intestinal epithelium caused by intestinal health issues, such as coccidiosis or dysbacteriosis, can reduce the bird's ability to absorb nutrients. For efficient digestion and absorption of nutrients, it is key to ensure the gastrointestinal tract is in optimal condition.

In both cases, undigested feed particles can reach the end-gut where they can serve as growth substrates for non-beneficial bacteria, further increasing the risk of health issues and reduced performance. The use of exogenous enzymes and probiotic bacteria in feed can contribute to the development of optimal conditions inside the gut ameliorating the impact of both challenges.

Xylanase degrades cell wall components of soluble and insoluble arabinoxylans present in the feed reducing digesta viscosity and releasing previously trapped nutrients (1, 2). Alpha-amylase increases the hydrolysis of starch (3, 4) improving starch digestibility (5, 6) while protease increases the hydrolysis of proteins including proteinaceous antinutrients (e.g. trypsin inhibitors) (7, 8, 9). The addition of these exogenous enzymes to the diet improves the nutritive value of the diet (9, 10, 11), allowing for improved performance indicators such as bodyweight gain, feed intake, egg mass, egg production and feed utilization (9, 10, 11).

Probiotics or Direct Fed Microbes (DFMs), such as Bacillus subtilis, are known to establish and maintain a beneficial microbial population in the gut of the host animal (12, 13, 14). Increased gut colonization by beneficial bacteria makes the gut environment less conducive to colonization by coliforms (15, 16, 17) reducing micro-organisms that may have a negative impact on animal performance (12, 14, 16). The beneficial bacteria aid in maintaining optimum villi height and crypt depth (17) ensuring the gut's ability to absorb nutrients for animal maintenance and growth (12, 15, 18, 19). Optimum gut function allows the animals to cope better with nutritional stress in the gut (12, 15, 17, 19-21).

Individually used exogenous enzymes or probiotics can improve bird performance however synergistic effects are observed when xylanase, amylase, protease and probiotic Bacillus sp. strains are combined. By creating an optimal environment for digestion through increased nutrient digestibility and a balanced gut microbial population, improved performance parameters such as increased bodyweight gain and nutrient utilization (22, 23, 24) can be achieved.

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POSTERS

P[01] Super Dose of Phytase in Broiler Diets

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Abstract

Commercially, the need for phosphorus in the rations is met by adding inorganic phosphorus sources such as monocalcium phosphate (MCP) and dicalcium phosphate (DCP) or phytase enzyme at 500-1000 FTU/kg levels. Concerns about reducing ration costs have led researchers to focus on alternative feed additives that can be used instead of costly inorganic phosphorus sources. For this purpose, the addition of phytase enzymes to high-dose broiler rations has been one of the most researched subjects in recent years. This review discusses the consequences of using high dose (>1500 FTU/kg) phytase in broiler diets.

Key phrases: Phytase, broiler, phosphorus, high dose

P02 The Effect of Using Herbal Extract as a Feed Additive for Turkeys on *Eimeira* Oocyst Count and Production Performance

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Abstract

Turkey coccidiosis is an important digestive system disease that causes performance losses. In this study, it was aimed to reveal the effect of herbal extract applied with feed on Eimeira oocyst numbers and performance in turkeys. The study was carried out on Nicholas 700 turkeys in two houses, which were determined as the experimental and control groups. In the experimental group, the herbal extract was used for 14 days along with the feed on the 70th day of production. Before using the herbal extract (on the 70th day) and after the use of the herbal extract (on the 91st day), 25 faeces were randomly taken from both the trial and control flocks from the crossover line, and collected and coded in 5 manure containers. The coded stools were delivered to the laboratory by providing cold chain (2-8°C) conditions. Eimeira oocyst counts were performed using the Modified Mac Master method. Eimeira oocyst counts were found to be between 150-300 ooc/gr in both the trial and control groups. The mortality rate of the turkeys in the experimental group was 8.5%, while the mortality rate in the control group was 15.7%. In addition, it was noted that the daily live weight gain was 3.5 g more in the experimental group. These results showed that the herbal extract can be used as an alternative to chemical anticoccidials.

Keywords: Coccidiosis, Eimeira, herbal extract, turkey

P03 Phylogenetic Analysis of Fowl Aviadenovirus 11 Detected in a Broiler Cluster

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Abstract

Diseases caused by Fowl Aviadenovirus in poultry industry cause serious economic losses worldwide. It causes different diseases in a wide age group in both breeders and broilers, and there are serotypes showing horizontal and vertical transmission. It is important to determine the circulating serotypes in the field for the protection and control of FAdV-induced diseases, especially in broiler breeding. In this study, the hexone gene region of FAdV-11 isolated from a broiler cluster in Ankara region was sequenced and its phylogenetic similarities with various strains previously reported in Turkey and the world were compared. It was observed that FAdV-11 obtained as a result of molecular comparisons showed 97.44% homology with FAdV-11 previously isolated in Turkey. It was also found to have high homology with Saudi Arabia, China and Ecuador when compared with the FAdV-11 serotype reported from various countries. As a result, it was seen that FAdV-11 serotype with different phylogenetic homologies from the previously reported FAdV-11 serotype in Turkey and in comparison with the strains reported from the world, the newly reported serotypes from different geographical regions could circulate.

P04 Does Combining Phytogenic Feed Additives and Esterified Short and Medium-Chain Fatty Acids Improve Broilers' Performance and Gut Integrity?

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The potent properties of plants have been recorded throughout history, and combining several phytogenic active substances in proper combinations can result in higher efficacy than the sum of the single ingredients and thus may lead to an improved health and growth performance of poultry. With the careful selection of different natural, plant-based ingredients and synergistic effects of short and medium-chain fatty acids (MCFAs), we can impact poultry performance by optimizing the gut functions with antimicrobial, antioxidative, and anti-inflammatory properties. To evaluate the effect of combinations of phytogenic feed additives (PFAs) included in the poultry feed to replace antibiotic growth promoters, the addition of Biostrong products was investigated for zootechnical performance and intestinal health parameters of broiler chickens subjected to moderate challenge conditions. A total of 1340 day-old Cobb 500 chickens was used for a trial on a test integration farm in Latin America with 4 treatments: a control group with no additives, a test group with virginiamycin at 200 ppm in feed, a test group with Biostrong® 1020 from d 1-48 at 75 g/MT and the last group with the Biostrong® program consisting of Biostrong® Forte from d 1-21 at 750 g/MT and Biostrong® 1020 from d 22-48 at 75 g/MT without any addition of growth promoters. To generate challenging conditions, reused litter was sprinkled daily with 1 L of water per pen. Performance parameters such as feed intake, body weight, and feed conversion ratio were monitored throughout the trial. At 28, 35, and 42 days, 22 birds per treatment were slaughtered, and samples were taken for histomorphometry and histopathology evaluation. At 28 days of life, a marked loss of integrity was observed in the duodenum, jejunum, and ileum of birds in the control group, significantly different from the rest of the treatments ($p \leq 0.05$). While at 35 and 42 days, the injuries were minor, not finding significant differences between treatments. A similar trend was observed in the inflammation of the duodenum, jejunum, and ileum. At 42 days, a more significant presence of inflammation was found in the jejunum-ileum of the control group and presented a different distribution of lesions with respect to the Biostrong® 1020 and Biostrong® Forte treatments. With respect to obtained results, the Biostrong® program shows a promising potential to improve broilers› growth performance and gut integrity under industrial conditions, presenting PFAs as an alternative to the use of AGP or, at certain ages, even better performance.

P05 Coloring Chicken Meat Cubes Using Anthocyanin-Rich Fruit Juices

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Abstract

In this study, chicken breast meat cubes were colored by vacuum impregnation (VI) technique using 4 different impregnation solutions (control, pomegranate juice, black mulberry juice, black grape juice) and then dried and transformed into a functional product. Color analysis, TPA (texture profile analysis) and sensory evaluation were performed on colored chicken cubes. Compared to the control group treated with salt solution, lower L^* and b^* values were determined in the samples treated with anthocyanin-rich fruit juices. The use of different fruit juices as marinade did not affect the textural properties of the dried samples. In sensory evaluation, chicken cubes impregnated with grape juice were more appreciated. As a result, it has been evaluated that colored and sensorially acceptable chicken meat cubes could be produced by vacuum impregnation technique.

Keywords: Black mulberry juice, pomegranate juice, grape juice, chicken meat, vacuum impregnation

P06 Effects of Free Access to Pasture Cultivated with Leguminous Forage Crops on Performance and Meat Quality in Slow Growing Broilers

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Abstact

In this study, access to the free-range planted with different legume forage crops was determined by performance (live weight, feed intake, feed conversion ratio, forage intake), meat quality (color, pH, STK, nutrient content, TBARS) and sensory analysis in slow growing broilers. effect was investigated. In the experiment, a total of 216 mixed sex slow growing broiler chicks (Hubbard Red Bro) were divided into 3 treatment groups (free-range planted with medicago sativa, trifolium repens or lotus corniculatus) with 4 replicates (9 females-9 males per group). Broilers had access to the free range area from day 21 until the end of the trial (day 72). While body weight, feed intake and feed conversion ratio were not affected by the treatments, forage intake was highest in the trifolium repens group ($P<0.05$). In all data obtained in meat quality and sensory analysis, gender was also analyzed as a factor. The KM and HP values of breast and thigh meat of the medicago sativa and trifolium repens groups were higher than those of the lotus corniculatus group ($P<0.05$). Breast meat pH value of trifolium repens group animals was higher than other groups ($P<0.05$). Likewise, breast meat pH and STK values of male animals were higher than females ($P<0.05$). While color values were not affected by forage type, breast meat b* value was higher in females and thigh meat L* value was higher in males ($P<0.05$). Breast meat TBARS (mg MDA/kg) was higher in females and affected by forage typeXsex interaction ($P<0.05$). Breast meat sensory analyzes were not affected by forage type and gender, while tenderness and taste and flavor were affected by forage typeXgender interaction ($P<0.05$). The results obtained in the current study show that medicago sativa, trifolium repens or lotus corniculatus forage crops can be evaluated and used as pasture crops for poultry without any negative impact on animal performance and meat quality.

Keywords: medicago sativa, trifolium repens, lotus corniculatus, free-range, slow growing broiler

P07 Antibacterial Activity of Herbal Extracts and Its Effect on Broiler Digestive System Microbiota

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Abstract

In recent years, the use of plant extracts, which are known to have antimicrobial properties, as drinking water and feed additives has been increasing. In this study, it was aimed to investigate the antibacterial activity of two products containing thyme and menthol extracts and their effects on broiler digestive microbiota. In the study, products containing plant extracts containing thyme and menthol were added separately by calculating the usage dose of the products on the liquid media inoculated in equal amounts from pure cultures of E.coli and S.Typhimurium, and the liquid media were incubated at 37 °C for 3 hours. Only bacterial cultures were added to control media. In the first and third hours, the mixtures were planted on Nutrient Agar and incubated at 37 °C for 18-24 hours and bacterial counts were carried out at the end of the incubation. According to the findings, the product containing thyme extract decreased the number of E.coli by 53.5% of the control group in the first hour, and by 75.4% in the third hour. It was determined that while S.Typhimurium strain decreased by 45.19% in the first hour compared to the control group, it decreased by 65.5% in the third hour. The efficacy of the product containing menthol extract, on the other hand, decreased the number of E.coli in the first hour by 8% of the control group and by 80% in the third hour. While S. Typhimurium decreased by 15.3% of the control group in the first hour, it decreased by 66.7% in the third hour. As a result of the microbiome analysis, the average Firmicutes and Actinobacteriota bacterial phyla were found to be higher in the experimental groups in which products containing thyme and menthol extract were given compared to the control group averages, while the Bacteroidales and Protobacteria bacterial phyla were found to be lower.

As a result, it was determined that products containing thyme and menthol extract have antimicrobial activity on E.coli and S.Typhimurium. In addition, it was observed that products containing thyme and menthol extract proportionally increased the beneficial bacteria in the digestive system microbiota.

Keywords: Antimicrobial activity, herbal extract, microbiome analysis

P08 Prevalence and Antibiotic Resistance Profile of *Yersinia Enterocolitica* in Chickens

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Abstract

Yersinia enterocolitica is the most important *Yersinia* species in terms of food safety, as an important food and waterborne enteric pathogen. In this study, it was aimed to determine the frequency and antimicrobial resistance profile of *Y. enterocolitica* in a total of 127 litter samples, which were obtained from broiler flocks between 2020-2021. For this purpose, bacterial isolation and identification from litter samples were carried out in 3 stages, namely enrichment in selective broth, cultivation in selective solid medium, and verification by biochemical tests. Antibiogram test of the obtained isolates was performed by Kirby-Bauer disk diffusion method. As a result of analysis, *Y. enterocolitica* was isolated with 8.66% (11/127) rate. As a result of antibiogram test, all isolates were succeptible to nalidixic acid, gentamicin, ciprofloxacin and imipenem. However, 70% or more resistance was detected to kanamycin, amoxicillin and ampicillin (respectively, %90,9, %81,1 ve %72,2). When all isolates were examined, 5 different multidrug-resistant (MDR) profiles were determined. The most common MDR profile (%45,45, 5/11) was determined as AM, AML, CF.

Keywords: Chicken, Yersinia enterocolitica, Antimicrobial resistant

P09 Alternative Poultry Production; Goose Production and Its Importance

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Abstract

This work was conducted to provide information about the importance of goose production as an alternative source of poultry meat. With the world population reaching 8 billion, different cheap, affordable and nutritive sources of animal protein are needed to meet the demand and the consumption rate of animal based protein. The most consumed meat in the world according to the OECD (Organization for Economic Development and Cooperation) statistical data was 36% pork, 33% poultry, 24% beef and 5% small ruminants. Out of the 43.5 kilograms of meat consumed per person in the world, 16.9 kilograms is poultry meat. In 2020 in the republic of Turkiye, out of the 36 kg of meat consumed, 21 kg of it was poultry meat. Chicken is the most consumed poultry specie in the globe but recently, the demand for other alternative poultry meats has been increasing. The production and consumption of goose, has gained a substantial interest in the UK, Canada, USA, China, Poland, France, Bulgaria and Russia. In recent years (2019), the population of goose, which reached 1.2 million in Turkiye, constituted 0.3% of the total poultry species in the country. Gooses are generally reared extensively in towns or villages and sometimes as part of family businesses. Gooses are normally reared for their meat, eggs, feathers and liver. Scientific studies on geese globally and in Turkiye are centered on breast muscle enhancement, carcass quality, and slaughter yield, behavior and nutrition. Efforts should be made to raise other poultry species, to direct people to different protein sources, to support goose production and to increase consumption.

Keywords; Alternative poultry, goose, production

P10 Effects of Trace Mineral Sources in Broiler Breeders

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Abstract

Broiler breeders are the parents of broiler chickens that produce chicken meat, and correct or incorrect practices in breeders nutrition directly affect the quantity, health and quality of chicks to be obtained. Trace minerals are defined as compounds that play a role in many important functions in animal nutrition, and whose deficiencies lead to significant health, productivity and performance losses. Inorganic trace mineral sources have been used in poultry nutrition for many years. As alternatives to these resources, organic trace mineral resources and hydroxy trace mineral resources, which have high bioavailability, have come to the fore and studies are being carried out on their use in animal nutrition. In this review, it is aimed to summarize the studies on different mineral resources used in broiler breeders.

P11 Radio Frequency Thawing and Its Effect on Proteolytic Changes Compared to Conventional Thawing Techniques in Frozen Block Chicken Breast Meat

Özge Erke, Zeynep Bacın, Eda Demirok Soncu, Eda Coşkun, Ferruh Erdoğdu

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Abstract

The aim of this study was thawing of chicken breast meat bulk (~9 kg in a 60×40×10 cm box) by radio frequency (RF) system and determination the effect of RF thawing on proteolytic changes as compared to conventional thawing methods performed at 4±0.1°C or 22±0.5°C. No significant differences were determined on the evaluated parameters in terms of RF thawing. On the other hand, thawing process was completed in 67 min by RF which shortened the thawing time by 5 and 43 times as compared to thawing at 22°C or 4°C. This finding proved that RF has been suggested as a promising thawing technique for time and energy savings on an industrial scale.

Keywords: Radio frequency thawing, low temperature thawing, chicken breast meat bulk, water holding capacity, protein solubility, protein oxidation, proteolytic changes

P12 Radio Frequency Thawing in Industrial-Scale Breast Meat: Optimization of Process Conditions and Its Effect on Quality Parameters

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Abstract

The purpose of this study was optimization of radio frequency (RF) thawing process conditions for commercially frozen chicken breast meat bulk and determination the effect of RF thawing on physicochemical, microbiological and lipolytic quality in meat as compared to conventional thawing methods. Results have been showed that RF thawing could be used succesfully in an industrial scale by reducing the thawing process time by 5 and 43 times as compared to thawing at 22°C and 4°C, respectively. Moreover, RF thawing could minimize the negative impact of freeze-thaw process providing the breast meat with lower cooking loss, softer texture, lower aerobic microbial count and lipid oxidation level as compared to thawing at 4°C, which is a fequently used thawing method in both industry and daily life.

Keywords: Radio frequency thawing, optimization, microbial quality, lipid oxidation, conventional thawing, texture, thawing loss

P13 Effect of A Product With Mycotoxin Biotransformation Properties On Performance of Broilers Fed With Naturally Fumonisins (FUM) and Deoxynivalenol (DON) Contaminated Diet

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Abstract

Mycotoxins are toxic contaminants naturally found in commodities and animal feed. Among them, Fumonisins (FUM) and Deoxynivalenol (DON) present a big challenge for poultry industry since both influence intestinal integrity of birds. Due to its structural characteristics, neither DON nor FUM, can be adsorbed or bound, therefore a technology which biotransforms both molecules is needed. The aim of this study was to evaluate the efficacy of a product which contains fumonisin esterase and a specific bacterium targeting mycotoxin biotransformation on performance of broiler chickens fed diets naturally contaminated by DON and FUM. A total of 400 one-day-old broilers (Ross 308; initial weight 40 g) were allocated into two experimental groups, ten replicates per group, 20 chicks per pen. Both groups received same feed contaminated by DON and FUM in phase 1, until 14d (FUM 2570ppb, DON 5050 ppb) and phase 2, from 15 to 35d (1180 ppb FUM, 3360 ppb DON). No negative control group with low contamination was feasible due to the natural contamination profile of the corn. The control group had no supplementation, while in treated group was added in a concentration of 1.5 kg/ton of feed of product. Performance results of body weight (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded for 35 days. It was possible to observe that control group had a numerically lower FI (-2,3%) when compared with treated group. While treated group showed a significantly higher ($P<0.05$) BWG by +7,5% than control. And, although not significant, treated group had better FCR (-4,9%) than control. The results indicate the addition of a product with mycotoxin biotransformation properties is effective to counteract the effects of a natural challenge by FUM and DON on performance of broilers.

Introduction

Mycotoxins are secondary metabolites produced by fungi which can be toxic and negatively influence health and performance of livestock. One of the major challenges in poultry industry is given by the fungi *Fusarium* sp. which produces a variety of mycotoxins, among them Fumonisins (FUM) and Deoxynivalenol (DON) are the most relevant for broiler production. Both compounds have effects over gut integrity and morphology, leading to a reduced absorption surface and a 'leaky gut` condition, which negatively influences zootechnical performance of birds (Antonissen et al., 2015; Andretta et al. 2011).

Ten years of analytical research (2012 – 2022) in Turkey and Middle East shown that FUM and DON are highly prevalent mycotoxins in corn, 92% and 44% respectively, and in poultry finished feed, 98% and 53% (DSM Mycotoxin Survey, 2022). Moreover, their contamination levels have an average of positives of 872 ppb for FUM and 6421 ppb for DON in poultry finished feed. The association between high prevalence and considerable contamination levels present a challenge for the local industry and necessity to protect animals against these toxic compounds.

Due to structural characteristics many mycotoxins, like DON and FUM, cannot be adsorbed or bound by binders (Sabater-Vilar *et al.* 2007; Fruhauf et al., 2012). Therefore, the aim of this study was to evaluate the efficacy of a mycotoxin detoxifier product containing a specific fumonisin esterase and a bacterium on performance broiler chickens fed diets naturally contaminated with DON and FUM.

Materials and Methods

A total of 400 one-day-old broilers (Ross 308; initial weight 40 g) were allocated into two experimental groups, ten replicates per group, 20 chicks per pen. Both groups received same feed contaminated by DON and FUM in phase 1 and 2 (table 1). No negative control group with low contamination was feasible due to the natural contamination profile of the corn. The control group had no supplementation, while in treated group was added in a concentration of 1.5 kg/ton of feed of the product

(Mycofix Select 5.0) which contains a fumonisin esterase enzyme (FUMzyme®) and a specific bacterium (BBSH 797).

Table 1. Experimental design and mycotoxin levels in feed (ppb)

	Control	Treated
Number of animals	200	200
Phase 1 (day 1 – 14)		
DON (ppb)	5,05	5,05
FUM (ppb) 2,570 2,570	2,57	2,57
Mycofix Select 5E (MSE 5.0) (kg/ton of feed)		1.5
Phase 2 (15 – 35)		
DON (ppb) 3,360 3,360	3,36	3,36
FUM (ppb) 1,180 1,180	1,18	1,18
Mycofix Select 5E (MSE 5.0) (kg/ton of feed)		1.5

Feed and water were offered *ad libidum* for all the periods. Performance results of body weight (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded for 35 days.

All data was subjected to statistical evaluation by means of ANOVA followed by Tukey-Test (IBM SPSS Statistics 19).

Results

The results have shown that control group had a numerically lower FI (-2,3%) when compared with treated group (Figure 1). While treated group showed a significantly higher (P<0.05) BWG by +7,5% than control (Figure 2). And, although not significant, treated group had better FCR (-4,9%) than control (Figure 3).

Figure 1. Feed intake (FI) (g/animal)

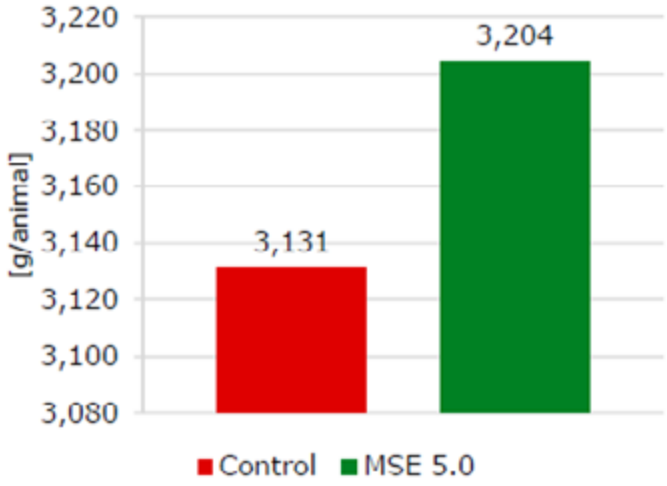


Figure 2. Body weight gain (BWG). a, b stat. sign. differences (P<0.05)

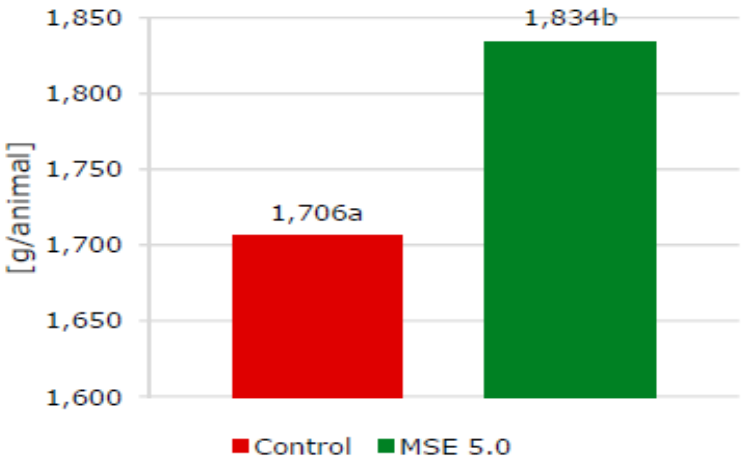
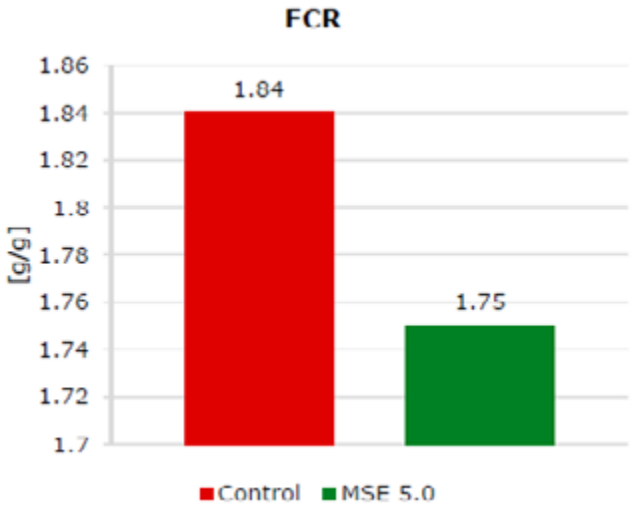


Figure 3. Feed conversion rate (FCR) (g/g)



Discussion

According to Andretta et al. (2011) and Kolawole et al. (2020) mycotoxins are able to negatively influence performance of broiler chickens. Previously, Greinier et al (2017) showed the efficacy of fumonisin esterase in detoxifying FUM in chickens and to maintain gut functions. Moreover, Awad et al. (2006) showed the effect of BBSH 797 in reducing DON effects on intestinal villi inn broiler chickens as well.

This study showed that the addition of a mycotoxin detoxifier product based in biotransformation can improve zootechnical parameters of protein deposition in broilers. Once DON and FUM were biotransformed into non-toxic metabolites, the negative effects on gut morphology and functionality didn't happen, allowing the animals to effectively absorb and utilize nutrients from the diet.

Conclusion

The results demonstrate the efficacy of mycotoxin detoxifier based in mycotoxin biotransformationto counteracting the effects of a natural challenge by FUM and DON and provide better performance for broilers chickens.

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P[14] Hemorrhagic Liver Syndrome in Broilers: Its Effect on Serum Parameters, Antioxidant Capacity, Liver Enzymes and Fatty Acid Profile of Liver

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Abstract

Liver is a vital organ with various functions in the body. The aim of the current project was to determine the incidence of levels of liver hemorrhagic syndrome (LHS) in broilers reared under commercial conditions and evaluate the effect of lesion scores on serum chemistry, antioxidant status, liver enzymes in serum, lipid, and protein oxidation level in liver, and fatty acid profile of liver. The incidence of liver lesion scored 0 to 5 was 12%, 31%, 33%, 15%, 6%, and 3%, respectively. Lower crude protein and higher crude fat contents were measured in 4 and 5 scored livers ($P< 0.05$). Livers with score 5 were significantly sensitive to lipid and protein oxidation as a result, the level of MDA, carbonyl, and sulphydryl were 82,8, 43,3, and 74,8% higher ($P< 0.05$) compared to livers with no LHS incidence. Serum's total protein in scores 4 and 5, and albumin in score 5 significantly dropped while uric acid and GGT in scores 4 and 5, total cholesterol and ALT in score 5 significantly raised ($P< 0.05$). The worst serum AST level was observed in scores 3,4, and 5 ($P< 0.05$). Compared to a normal liver, serum SOD, GSH PX, and total antioxidant showed a significant ($P< 0.05$) rising trend moving from score 1 to 3 while the same factors demonstrated significant reduction in livers scored 4 and 5. The results revealed that livers scored 4 and 5 had the highest ($P< 0.05$) level of total saturated fatty acids. Besides, total MUFA, n6, n3, and PUFA levels significantly decreased in livers with 4 and 5 LHS scores. Briefly, the current study deducted that high levels of LHS scores may affect the physicochemical and oxidative quality of livers and blood serum; however, more studies are needed to assert an accurate and explicit judgement.

Keyword: broiler, liver hemorrhage, serum parameters, antioxidant capacity, liver enzymes, fatty acid profile

Introduction

Liver is digestive system's accessory organ with many major functions and responsibilities such as synthesis, metabolism (fat, carbohydrate, protein, vitamins, and minerals), excretion, and detoxification processes. It has a very important role in digestion and metabolism, regulating the production, storage, and releasing of carbohydrates, lipids, and proteins (Denbow, 2000). So, whether this vital organ functions normally or not demands great attention and bears an essential spot in research. Fatty liver hemorrhagic syndrome (FLHS) is a metabolic problem in commercial laying hen flocks with high levels of prevalence and occurrence. Sometimes, FLHS causes death in high producing laying flocks. The problem can also be seen in commercial broiler flocks which is mainly known as liver hemorrhagic syndrome (LHS). Nutrient density, antinutritional factors (e.g., NSP, Phytate), toxic substances (e.g., various mycotoxins), chemicals (e.g., heavy metals), and drugs (e.g., antibiotics) can affect normal function and health status of the liver (Akers and Denbow, 2013; Zaefarian et al., 2019).

Materials and Methods

The incidence of liver lesions was observed through lesion scoring, by collecting samples from a broiler integration in Türkiye, from June 2019 to July 2022. The livers were examined for the presence of lesions and hemorrhagic scoring. Liver lesions were graded on a scale from 0 to 5 based on the method recommended by Shini et al. (2019). The incidence of each score were as 12%, 31%, 33%, 15%, 6%, and 3% respectively from 0 to 5 scores. All raw materials used in feed formulation were analyzed to determine the existence and levels of probable mycotoxin contamination. Lab analysis included proximate composition, lipid and protein oxidation, and fatty acid profile of liver, and blood biochemistry and antioxidant status of each liver score.

For laboratory analysis, a total of 1,000 birds fed with the same diet composition (Ross 308 broilers) were separated from 12,000 birds aged 41 days, after slaughter from the same flock of a commercial slaughterhouse. Then, the selected slaughtered birds were lesion scored for the liver hemorrhage. After scoring, 20 livers were separated for each score, freezed under -20˚C until

further analysis. Samples were packed on ice and transported to the Ankara University Animal Science laboratory to be analyzed.

Blood samples were collected from basilic vein of birds (20 birds per score) by venipuncture in serum vacutainers with gel as clotting activator. After clotting, sera were separated by centrifugation at 4500 rpm for 15 minutes. Separated sera was transferred into Eppendorf tubes for further analysis.

Results

The lowest fat and the highest protein amounts were measured in scores 0 to 3 while the opposite trend occurred for the livers with score 5 which exhibited the highest fat and lowest protein contents (table 1; P< 0.05). Livers with score 5 were significantly sensitive to lipid and protein oxidation, and the level of TBARS, carbonyl, and sulphydryl were higher by 82.8, 43.3, and 74.8% (table 2; P< 0.05) in score 5 compared to livers with no LHS (table 2). The findings indicated no significant differences in serum total protein and albumin level in birds with score from 0 to 4 but the birds with score 5 had the lowest total protein and albumin (P< 0.05; Table 3).

Table 1. Effect of the severity of liver hemorrhage on proximate analysis of liver

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	P-value
Liver Weight, % of BW	2,266± 0,020	2,298± 0,056	2,264± 0,082	2,263± 0,117	2,282± 0,09	2,333± 0,08	0,553
Dry Matter, %	24,194± 1,189	24,353± 0,44	24,363± 0,569	24,147± 1,015	24,338± 1,183	24,066± 1,157	0,983
Crude Fat, %	11,918± 0,864 b	12,054± 0,738 b	12,108± 0,742 b	12,077± 0,623 b	12,948± 0,47 ab	13,395± 1,029 a	0,002
Crude Protein, %	68,761± 0,470 a	68,822± 0,316 a	68,889± 0,574 a	68,744± 0,46 a	67,603± 0,713 b	67,564± 0,348 b	0,0001
Crude Ash, %	5,643± 0,497	5,718± 0,383	5,831± 0,765	5,726± 0,5	5,474± 0,49	5,813± 0,547	0,726

[a,b] Means within the same raw without common superscripts are significantly different ($P < 0.05$)

Table 2. Effect of the severity of liver hemorrhage on liver lipid and protein oxidation

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	P-value
MDA	1,732± 0,333 b	1,807± 0,564 b	1,893± 0,503 b	1,724± 0,169 b	2,179± 0,435 b	3,166± 1,039 a	0,0001
Carbonyl	50,16± 18,75 b	50,890± 3,4 b	52,000± 4,78 ab	53,070± 3,32 ab	62,970± 5,5 ab	71,890± 3,86 a	0,018
Sulphydryl	7,409± 2,238 b	7,596± 2,689 b	8,820± 2,276 b	10,13± 4,69 ab	11,020± 4,86 ab	13,170± 5,86 a	0,0001

[a,c] Means within the same raw without common superscripts are significantly different ($P < 0.05$)

Table 3. Effect of the severity of liver hemorrhage on serum biochemistry, liver enzymes and antioxidant capacity

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	P-value
Total Protein	3,394± 0,633 a	3,498± 0,535 a	3,455± 0,774 a	3,022± 0,613 ab	2,772± 0,496 b	2,252± 0,377 c	0,0001
Albumin	1,536± 0,205 a	1,521± 0,214 a	1,419± 0,225 ab	1,305± 0,133 bc	1,297± 0,187 bc	1,201± 0,115 c	0,0001
Uric acid	3,90± 0,592 b	3,771± 0,524 b	3,743± 0,469 b	4,296± 0,456 b	5,992± 0,979 a	6,577± 0,657 a	0,0001
Total Cholestrol	127,56± 6,64 c	127,680± 6,77 c	127,860± 4,13 c	131,530± 4,84 bc	133,680± 3,93 ab	136,400± 3,24 a	0,0001
AST	274,06± 41,30 b	275,200± 56,3 b	278,500± 50,9 b	337,680± 24,05 a	351,850± 27,29 a	358,850± 29,79 a	0,0001
GGT	13,40± 3,289 b	13,550± 4,66 b	13,903± 4,348 b	17,060± 4,52 ab	18,560± 4,94 a	20,169± 3,99 a	0,0001
ALT	17,436± 3,058 c	17,477± 3,331 c	18,862± 3,604 c	19,572± 3,163 bc	22,115± 3,76 ab	24,123± 3,426 a	0,0001
SOD	92,59± 4,69 c	92,600± 4,7 c	102,740± 7,33 b	113,860± 9,46 a	77,700± 7,07 d	76,350± 4,77 d	0,0001
GSH PX	669,4± 33,51 b	670,030± 37,02 b	746,060± 34,08 a	765,520± 27,82 a	616,120± 40,28 c	602,510± 33,3 c	0,0001
Total antioxidant	6,491± 1,386 b	6,491± 1,661 b	8,119± 1,393 a	8,442± 1,476 a	4,563± 1,255 c	4,356± 0,992 c	0,0001

[a,c] Means within the same raw without common superscripts are significantly different ($P < 0.05$)

Conclusion

Briefly, the current study showed that high levels of LHS scores may affect the physicochemical and oxidative quality of livers and blood serum; however, more studies are needed to assert an accurate and explicit judgement.

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Table 4. Effect of the severity of liver hemorrhage on fatty acid profile of liver

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	P-value
C14:0	0,345± 0,019	0,350± 0,018	0,354± 0,029	0,357± 0,035	0,351± 0,016	0,375± 0,032	0,461
C16:0	21,776± 0,952 c	21,737± 0,971 c	21,91± 1,012 c	22,155± 1,941 c	25,615± 2,152 b	28,582± 1,72 a	0,0001
C18:0	20,744± 1,259 b	20,595± 1,003 b	20,891± 1,722 b	20,203± 1,329 b	22,539± 1,267 a	22,492± 2,065 a	0,015
C16:1(n-7)	1,643± 0,112	1,654± 0,185	1,561± 0,165	1,691± 0,122	1,567± 0,282	1,719± 0,14	0,419
C18:1(n-9)	21,455± 0,932 a	21,232± 0,647 a	21,407± 1,351 a	21,092± 0,957 a	20,523± 1,507 a	18,662± 0,789 b	0,0001
C18:1(n-7)	0,072± 0,017	0,073± 0,011	0,067± 0,018	0,075± 0,018	0,074± 0,013	0,076± 0,01	0,843
C20:1(n-9)	0,506± 0,084	0,486± 0,055	0,522± 0,096	0,493± 0,071	0,458± 0,09	0,482± 0,086	0,739
C18:2(n-6)	18,510± 1,210 a	18,292± 1,006 ab	18,185± 0,949 ab	18,252± 1,43 ab	16,513± 1,01 bc	15,961± 1,629 c	0,0001
C20:4(n-6)	11,801± 0,821 a	11,518± 0,668 a	11,035± 0,709 a	11,484± 0,927 a	9,034 ±0,914 b	8,728± 1,781 b	0,0001
C22:4 (n-6)	0,609± 0,022 a	0,596± 0,053 a	0,578± 0,089 a	0,609± 0,16 a	0,445± 0,13 ab	0,382± 0,07 8b	0,0001
18:3(n-3)	0,408± 0,051 a	0,390± 0,027 ab	0,385± 0,037 ab	0,396± 0,079 ab	0,329± 0,052 bc	0,302± 0,043 c	0,0001
C20:5(n-3)	0,571± 0,056 a	0,553± 0,068 a	0,516± 0,039 a	0,548± 0,049 a	0,352± 0,041 b	0,321± 0,049 b	0,0001
C22:5(n-3)	0,545± 0,083 a	0,542± 0,048 a	0,525± 0,053 a	0,539± 0,076 a	0,398± 0,058 b	0,359± 0,056 b	0,0001
C22:6(n-3)	1,636± 0,098 a	1,629± 0,109 a	1,621± 0,309 a	1,573± 0,235 a	1,276± 0,145 b	1,107± 0,097 b	0,0001
SFA	42,865± 1,343 c	42,682± 1,337 c	43,156± 1,579 c	42,714± 2,249 c	48,505± 1,929 b	51,449± 1,823 a	0,0001
MUFA	23,677± 1,328 a	23,445± 1,706 a	23,558± 1,533 a	23,351± 1,154 a	22,623± 1,454 b	20,939± 1,255 b	0,001
n6	30,921± 1,340 a	30,406± 1,297 a	29,798± 0,917 a	30,346± 1,929 a	25,992± 1,311 b	25,069± 1,935 b	0,0001
n3	3,159± 0,175 a	3,114± 0,169 a	3,047± 0,266 a	3,056± 0,261 a	2,355± 0,136 b	2,089± 0,102 b	0,0001
PUFA	34,080± 1,391 a	33,519± 1,339 a	32,845± 1,101 a	33,401± 2,018 a	28,347± 1,358 b	27,158± 1,956 b	0,0001
n6to3n	9,796± 0,984 c	9,787± 0,619 c	9,831± 0,727 c	9,982± 0,919 bc	11,061± 0,726 ab	12,018± 1,025 a	0,0001

[a,c] Means within the same raw without common superscripts are significantly different ($P < 0.05$)

P[15] Microbiological Monitoring of Commonly Found Bacterial and Fungal Contaminants in Chicken-Based Ready-to-Eat Fast Foods

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Abstract

Chicken is a supreme source of essential proteins which are important for the overall health and well-being of human beings. Due to the drastic change in the lifestyle of people, the dependence on ready-to-eat (RTE) foods is increasing day by day all around the world. All along with that, chicken is one of the most frequently used white meat in ready-to-eat food items such as chicken burgers, sandwiches, chicken rolls, chicken samosa, chicken kebabs, and other products. RTE foods which include chicken can harbor a variety of microorganisms that cause food poisoning and other life-threatening gastrointestinal infection complications. The main sources of the microbes in the RTE food items may vary from the improper handling of the chicken meat during the processing, improper cooking, unhygienic environment, sick restaurant personnel, use of inferior quality chicken meat, eggs, and green vegetables used in the fast food items. Another important reason is the cross-contamination of the carcass during the slaughtering process from the intestinal microflora of the chicken. Several microbes tend to grow in frozen RTE chicken items such as *Listeria monocytogenes* and cause a great number of serious infections in consumers. This is crucial to have a deep insight into the food-borne infections caused by the pathogenic bacteria and fungi present in the RTE food items. This meta-analysis was aimed to monitor the bacterial and fungal contaminants in chicken-based RTE fast foods and important strategies to deal with these problems to control the food-borne illness.

Keywords: Ready-to-eat chicken food, Microbes in fast food, food poisoning, white meat, Listeriosis

Introduction

During this fast age of technology, people are looking for convenient and quick food options. The inclination towards the ready-to-eat (RTE) food has gotten more attention because of hard working routines, single parent families and mostly among the youngsters. RTE food items are easy to serve and they can be readily eaten (1, 2).However, RTE fast foods are served quickly and require very little preparation time. The RTE foods are mostly made up of chicken and chicken-based products (1,4,8)

The chicken burgers, nuggets, pizza, sandwiches and along with other frozen chicken food items are very popular around the world (7). The consumers always desire to have good quality of RTE food items with appetizing organoleptic characteristics (smell, taste, appearance) etc. However, these food items are the suitable medium for the growth of number of microorganisms including bacteria and fungi (4,5,6).

The important sources of contamination of these microorganisms include the unhygienic processing of chicken, poor sanitation (during the manufacturing and at the restaurant), lack of personal hygiene of the personals, usage of bad quality/ spoiled chicken, and use of improperly washed green vegetables, low cooking time and much more (9,21). The slaughtering process of the chicken also causes contamination of the carcass with the intestinal microflora (11,12) The consumption of such RTE food items particularly from the street vendors causes a number of public health related problems in the people including food poisoning, diarrhea and severe gastrointestinal complications (4, 9,12).

The microbial contamination of the RTE foods can occur at any step including the preparation, processing, transportation, storage and serving (The important species of bacteria which are frequently found in the ready to eat (chicken-based) food items are *Salmonella*, *Staphylococcus aureus, Eschrichia coli, Campylobacter jejuni, Listeria monocytogenes, Clostridium botulinum, Yersinia enterocolitica, Bacillus cereus* and *Vibrio parahaemolyticus* (17,18,19). The important fungal species include *Aspergillus* species (*A.flavus, A.niger*), *Rhizopus spp, Mucor spp, Fusarium spp, Pencilliumspp, Trichoderma spp and* also some yeasts

(1,2,3,23).The key sources of these fungi are the environment, the vegetables and water used to make them (6,10).

Listeria monocytogenes is one of the major bacterial pathogens which cause food poisoning in human beings. The higher levels of *Listeria monocytogenes* have found to be present in ready to eat food items particularly made up of chicken (4,5,20). The growth of *Listeria* has also been seen in the chicken salads stored at the lower temperature showing the ability of this pathogen to show growth kinetics at refrigerator temperatures. Last but not least, the partially cooked chicken based food has quantities of the pathogenic microorganisms (5,15,22).

The hygienic practice during the processing, preparing, transporting and storing of the ready to eat food items decreases the risk of the pathogenic microorganisms (6,7,24). The monitoring of microbiological safety is very important in each step of production, processing, transportation, storage and serving of the ready to eat food items. All along with that, the microbial counts (aerobic bacterial counts) are very crucial for the food safety and protection of the consumers (4,5,16).

Microbial analysis of (RTE) food items : The RTE food items are prone to microbial contaminations during the preparation and the serving steps (1,2,13). The following table indicates the presence of bacterial and fungal contaminants in the chicken based food items by total viable count (TVC) and aerobic plate count (APC) (Table.3.1)

Table 1. Surveillance of different bacterial and fungal pathogens in different Ready to eat chicken based foods.

Type of (RTF)	Bacterial contaminants	Fungal contaminants	Reference
Chicken samosa	*Salmonella spp* *Staphylococcus spp,* *E.coli*	---	Sabuj et al. (1)
Chicken burgers	*E.coli* *Staphylococcus aureus* *Salmonella*	*Aspergillus spp*	Soher et al. (2)
Chicken sandwiches (Chicken fillet, minced chicken fillet, Chicken sausages)	*Coliforms,* *Bacillus cereus* *Salmonella spp* *Staphylococcus aureus*	-----	Christison et al. (13)
Chicken rolls	*Staphylococcus aureus* *E.coli*	----	Sultana et al. (3)
Chicken nuggets	*E.coli* *Staphylococcus aureus* *Campylobacterjejuni*	----	Soher et al.(2)

RTE: Ready to eat food

The table indicates that the surveillance of bacterial contaminants is quite greater than the fungal contaminants. This is clear that the *Salmonella spp, E.coli, Staphylococcus aureus, Coliforms, and Campylobacter jejuni* are the common contaminants of the RTE chicken based food and pose greater health risk to human beings.

Important parameters to handle the ready to eat food items **:** The following parameters affect the microbial load in the ready to eat (RTE) fast food items. These parameters are very much important to ensure the microbial safety of the ready to eat food items and also control the foodborne illness in the human beings (1, 2,10).

1) Type of ready to eat food (Preparation method)
2) Storage conditions and types(Enclosed area, open area)
3) Type of utensils used to serve the ready to eat food (Shared utensils, single use utensils)
4) Reheating the ready to eat food before serving (Microwave heating)
5) Health status of the food handlers and handling conditions (protective gloves, bare handed handling)
6) Management of the leftover ready to eat food (either disposal, reuse on the following day)
7) The education level of the handlers (trained about the food preparations, handling and microbiological safety or not).
8) Cross-contamination of the carcass during the slaughtering process from the intestinal microflora of the chicken. Salmonella is the most common bacteria that contaminate the carcass during the slaughtering.

Analysis of microbial risk assessment :Microbial risk assessment has four important steps which have been mentioned below step by step.

1) **Hazard identification**: This includes the presence of hazardous microorganisms which are frequently found in the ready to eat fast foods. According to the surveillance, *Salmonella, E.coli* and *Staphylococcus spp*are commonly found in the fast food items and considered as model QMRA (quantitative microbiological risk assessment) organisms (6,14).

2) **Hazard characterization**: This explains the worst health related effects produced due to intake of microorganism. The dose-relationship model is usually used to find the probability of the pathogenic microorganism and the infection caused by them (5,7,8). However, the probability of infection caused by a particular microorganism present in the ready to eat fast food can be calculated by using the following formula:

$$P(d)=1-e^{rd}$$

P= probability

D= dose of microorganism consumed per person in a day (cfu)

R= infectivity constant (varies among microorganisms)

D= Final dose of microorganism consumed per person per serving of the food.

3) **Exposure assessment**: This step highlights the exposure of the person with the microorganism through the frequency of the ready to eat food consumed to a specific period of time. For example, daily consumption, weekly consumption or monthly consumption of ready to eat foods (5, 7).

4) **Risk characterization:** This step is the combination of the all the previous scenarios. It includes the overall probability of the infection due to the exposure to the hazardous microorganism in every scenario (4,5).This is used to find the probability of the infection and the number of days of the exposure in a year.

$$\mathbf{P_{ann} = 1-\{1-P\,(d)\}^{n}}$$

N= number of days of exposure in a year

Conclusion

Ready to eat food items are easy to serve and pose high threat of food borne illness. The important microbial contaminants found in the chicken-based (RTF) are *Salmonella, E.coli* and *Listeria spp.* There is diverse variety of cross- contamination microbes in the food such as the personal hygiene of the manufacturers, equipments, the microbiological quality of chicken(monitoring of the microbiological safety of the carcass) and vegetables used in the RTF, Transportation, storage conditions and much more. This is important for the manufacturers to strictly obey the rules of as per the instruction of HACCP and FAO. This is also important to check the sanitary indicators microbes along with the specific food borne microbes of the ready-to-eat food items to ensure the microbiological safety. All along with that, the vendors and the food handlers should get the proper education on food hygiene and safety to limit the food-borne infections in the human beings.

HACCP: Hazard analysis and critical control point, FAO: Food and agriculture organization

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P[16] The Effect of Isoquinoline Alkaloids (IQs) on Broiler Performance Parameters

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Abstract

Macleaya cordata is a plant of the family *Papaveraceae*, containing mainly isoquinoline alkaloids (IQs). It is known to be used safely in many animal species today. In this study, the effects of adding IQs to broiler drinking water on Feed Conversion Ratio (FCR), European Production Efficiency Factor (EPEF), Average Daily Gain (ADG), Final Body Weight (FBW) and Survival Rate (SR) in broiler flocks were investigated.

Results and Conclusions

This field study shows that the use of IQs in broiler chickens has significant effects on FCR, EPEF, ADG, FBW and SR. Considering our findings, it was observed that the use of IQs, especially in the early stages, increased the livability. In addition, while lower FCR was obtained in the groups where IQs was used compared to the control group, higher ADG and FBW were obtained. At the same time, it was deemed appropriate to send the trial groups to slaughter one day earlier than the control groups. The findings obtained by Lee et al. (2015) and Liu et al. (2020) is supported by research studies. Similarly, Vieira et al. (2008) reported that IQs supplementation significantly improved the body weight of broilers at the early stage. At the same time, IQs modulates feed intake by reducing the decarboxylation of aromatic amino acids in the intestinal lumen, balancing intestinal pH and affecting the Trp-Ser pathway. In this way, it promotes growth in broiler chickens by improving the digestion and absorption of proteins (Li et al., 2018)

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P17 Fearfulness Response of Local Broiler Dam Line and Commercial Broiler Chicken Genotypes

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Abstract

In this study, fearfulness of two meat type chicken genotypes, one of the local broiler dam line and commercial broiler, were measured through tonic immobility (TI) and inversion tests. A total of 48 chickens, 24 from each genotype, were used in fear tests at slaughter age. Genotypes significantly differed in their responses to fear tests ($P<0.05$). Broilers from fast growing commercial genotype had longer TI duration and percentage of birds that had been induced TI was higher as compared to local genotype. In the inversion test, the number and duration of wing flapping did not change depending on the genotype, but flapping intensity was less in the local genotype ($P<0.05$). According to the findings under the experimental conditions, it was concluded that the fearfulness of the slow growing local broiler dam line was lower than the fast growing commercial genotype.

Keywords: Broiler, pure line, fearfulness, tonic immobility test, inversion test

P[18] Use of Herbal Additives in Poultry Diets as a Sustainable Method Against The Negative Effects of Heat Stress on Poultry Production and Meat Quality in Global Warming Conditions

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Abstract

The negative effects of global warming can be seen in social and economic areas. Higher temperatures, which generate heat stress, can be create serious problems compromising animal welfare, productivity, and product quality in poultry industry especially in summer seasons. The studies on this topic demonstrated that all types of heat stress (acute or chronic) have negative effects on the poultry meat quality, and they cause great economical loss. At the other hand, there are some methods applied for decreasing the negative influences of heat stress. Recently, some decreasing effects of plant based natural additives included in poultry diet against to the heat stress are reported in some studies. This strategy is suggested as an innovative and sustainable method concerned in animal husbandry. The objective of this review was to investigate the influences of heat stress, which became more serious with the rise of global warming, on the poultry meat quality parameters and to inform about the use of some plant-based additives for reducing the negative effects of heat stress, being as an innovative and sustainable method.

Keywords: Global warming, heat stress, poultry meat quality properties, sustainability, plant-based additives.

P19 Effect of Eubiotics Supplementation to Quail Diets on Performance, Small Intestine Histomorphology and Cell Proliferation

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Abstract

The aim of the study is to examine the effects of the addition of probiotic and active ingredient mixture+benzoic acid separately and together on performance, small intestine histomorphology and immunohistochemistry in quail diets. In the study, a total of 200 quails were divided into 1 control and three experimental groups, and each experimental group was divided into 5 subgroups consisting of 10 quails. Experimental group I: 18g/ton probiotic (E. faecium); Experimental group II: 300 g/ton of active ingredient+ benzoic acid mixture (thymol, eugenol, piperine, benzoic acid) and Experimental group III: probiotic with active ingredient and benzoic acid mixture were added together. At the end of the study, while there was no difference between the control and experimental groups in terms of live weight, live weight gain, feed consumption and feed conversion ratio ($P>0.05$), the carcass yield was determined in the group with the highest probiotic supplementation ($P\leq0.001$). Villus height, crypt depth, villus surface area and total mucosal thickness were evaluated histomorphologically in duodenum and ileum segments, which are among the parameters we used to evaluate intestinal health. The results of the study showed that probiotic and active ingredient + benzoic acid mixture added to quail diets caused significant increases ($P<0.05$) in intestinal histomorphology. In addition, PCNA expression density and proliferation index were determined by immunohistochemical method in order to determine cell proliferation in the duodenum and ileum. The addition of probiotic and active ingredient+benzoic acid mixture to the quail diets in the experimental groups caused significant increases in cell proliferation ($P<0.05$). As a result, the functionality and health of the small intestinal epithelium and mucosa, where nutrients are exposed to intense absorption, are very important for the general health and performance of the animal. In this study, we can say that the addition of probiotic and active ingredient+benzoic acid mixture, which is one of the eubiotic additives, to quail diets has positive effects on intestinal health.

P20 The Sustainable Approaches in Industrial Poultry Production: Nutrition

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Abstract

Todays' industrial poultry production generates the numerous ecological and human health footprints which are contribute to global matters such as rising temperatures, pollution of water resources, bacterial resistance against to antibiotics etc. Due to these problems, there serious attempts to adapt sustainable approaches to poultry production. Since the nutrition basically contributes in poultry production and its reveres impacts, to recognize and apply sustainable nutrition strategies could be beneficial related to above mentioned issues. Some of these strategies are suggest the replacement of conventional feed raw materials by ecologically low-cost feedstuffs with or without applying enzymes. At the same time, the reduced nutrients excretion and financially profitable are another benefits. To use feed additives such as probiotics, prebiotics and organic acids could answer to concern about antibiotic resistance. While another sustainable practices such as ideal protein concept, phase feeding and accurate calculation of nutrients requirement would lead to decline in nitrogen and phosphorus excretion.

Introduction

The sustainability is defined as meeting the needs of the present generations while preserving it to meet the needs of future generations. Sustainability is comprised of three pillars e.g. social, environment, and economic. This implies that any practice should be socially equitable, environmentally bearable, and economically viable. Poultry nutrition has evolved over the years with many practices that are not sustainable from the perspectives of any of these three pillars. The excretion of nutrients and gases to the environment is contributing to the environmental degradation and potential hazards. These involve the emission of greenhouse gases (GHGe) and excretion of nitrogen (N) and phosphorus (P). Moreover, the widely application of antibiotics as growth promoters in farm animals has led to the microbial resistance and increased public concerns towards antibiotic resistance and drug residues.

The efficient use of conventional feedstuffs and finding of new or alternative feed resources are among the sustainable approaches to animal nutrition. These include the formulation of poultry diets on ideal protein concept, use of enzymes to increase the digestibility of nutrients, and to reduce the N and P excretion. Some strategies have been employed to reduce the excretion of N and P, especially in poultry diets. Phase-feeding and separate sex feeding are among the sustainable practices of feeding management aimed to minimize the N and P excretion, and wastage of nutrients. The supplementation of feed by prebiotics, probiotics, symbiotic, and organic acids as feed additives can help to boost the poultry immune system and decrease the antibiotic usage. The aim of the present review is to discuss the poultry production impacts and sustainable practices in poultry nutrition in detail.

Todays' Poultry Production Advances and Nutritional Related Adverse Impacts

The chicken genome was sequenced in 2004, before any other farm animals [1]. This has allowed scientists to breed chickens to better grow in an industrialized setting. The most common industrial chicken breeds today are hybrids that are produced by crossing several breeds through a natural selection. As the consequence, the overall trend has been a phenomenal increase in biological productivity for both layers and broiler chickens. As an illustration, between 1935 and 1995, the mean slaughter weight of commercial broilers enhanced by roughly 65%, while the required rearing period decreased by more than 60% and the feed required to produce a pound of broiler meat declined by 57% [2]. On the contrary, hens bred for higher levels of egg production have fragile bones and fast growing broilers show leg, cardiovascular and respiratory disorders [3].

The World Health Organization (WHO) and many other public health bodies consider antibiotic resistance a global public health crisis. Antibiotics are medically essential drugs, responsible for saving millions of lives since they were developed. Antibiotics ability to promote growth rate of farm animals can boost the feed efficiency. Depressing intestinal normal microbiota is a possible mechanism to growth promotion by using antibiotics in animal feeding that lead to increased nutrient usage and a decrease in the maintenance charges of the gastrointestinal tract [4]. When bacteria are repeatedly exposed to

particular doses of an antibiotic, those resistant to the antibiotic will survive and proliferate while the rest were removed, resulting in a new bacteria population that is resistant to the antibiotic.

Regarding to animal welfare, the industrial rising of broiler chicken and turkey with high stock density, some nutritional factors such as non-starch polysaccharides (NSPs), protein source and high nutrient density cause wet litter which is leading foot pad dermatitis (FPD), hock burn and breast burns-lesions [5, 6]. Furthermore, ammonia is an acrid and dangerous gas formed when chicken manure breaks down. Inside the chicken house, it can reach levels high enough to be harmful to the birds, especially when the litter gets wet or has not been changed between flocks. Problems that elevated ammonia levels can cause include; eye problems, compromised respiratory system which can lead to increase rates of infection, and skin inflammation and infection on their feet and breast which are lead also to the performance decline [7, 8].

The poultry feed is designed to grow the chickens as quickly or product more egg as possible. It is made mostly of grains, especially corn and soybeans, supplemented with vitamins, minerals and enzymes. Environmentally, the conventional feed grains are a high-cost include: they are genetically modified, grown with chemical pesticides and fertilizers that can run off into groundwater sources and depend on fossil fuels for planting, harvesting and transport. Moreover, the significant amount of nutrients pass poultry digestive tract to manure due to typically highly concentrated feed formulations.

The high levels of the greenhouse gasses such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are also associated with industrial poultry meat and egg production [9]. The major contribution of GHGe from poultry meat production is generated by feedstuffs production (firstly corn 53%, followed by transportation of inputs 23%, on-farm energy utilization 12%, and N_2O emissions from the litter 11%). Alike to meat type facilities, GHGe from egg farm operations come primarily from feed production, on-farm energy use, N_2O from the poultry litter and fuel usage. While the post-farm emissions account for just 24% of total emissions [10].

In term of water consumption, poultry meat has a relatively small water footprint, as compared with other livestock, because of their mass production and small size. Conventional chicken production requires less than one- third the water needed by beef besides that on a per-grams-of-protein basis, egg production requires less than 85% of the water required by beef [11]. Nevertheless, the industrial poultry production can be pollutant foe water resources. The chicken manure is especially high in both P and N. When it rains, the excess nutrients and drug residues run off the fields into rivers and leak into groundwater sources. Manure that leaks into groundwater or runs off into surface water carries excess N and P, which can contaminate drinking water, reduce dissolved oxygen level of water, or cause algal blooms and make serious hazard for aquatic species [12].

Sustainable Poultry Nutrition

The aim of sustainable agriculture is to meet mankind's requirements (food and textile) in the present without making risks to possibility of next generations to meet their own needs. Since poultry products (meat and egg) have become favourite food as well as protein source of our daily meal, the sustainable poultry production is not possible without finding solutions for poultry nutrition sustainably. Because the most industrial poultry production adverse impacts are generated by nutrition related issues. The major part of poultry diet is consisted of corn and soybean meal in intensive poultry production. As feed constitutes 60-70% of the total cost of rearing, animal nutritionists inevitably have attempted to substitute the new feed formulation which are more efficient diets and decrease production costs and environmental adverse impacts [13]. Regarding to continuously increasing costs of conventional feed raw materials used for poultry nutrition like corn, soybean meal, fish meal etc. as well as environmental concerns, each new raw material for poultry feeds need to have consistent appropriate nutrient composition, lesser ecological footprint, a low cost per unit of key nutrients and be destitute of both food safety matters and anti-nutritional ingredients [14]. At the same time, the nutritional composition values and digestible rate differences of corn and soybean that are planted in different ecological zones are another problem which lead to less effective poultry production.

The protein composition of crops, as the widely used protein item of poultry feed, in many cases have low amounts of lysine, methionine, tryptophan and threonine [15]. To solve this limitation some insect species can be utilized in which also help to achieve ideal protein concept in poultry diet. Insects are a wealthy in terms of protein amount (vary from 40-60%), essential amino acids and fat. Dietary use of mealworm has shown high digestibility of nutrients, as the alternative raw material for soybean meal and fishmeal [16]. On the other hand, it should be noted that nutrient concentrations of insects may vary by their life stage, rearing condition and the composition of the growth substrate utilized.

The public concerns due to antibiotic resistance lead to increasing interests to antibiotic alternative growth promoters such

as probiotics, prebiotics, organic acids, etc during last decades. Probiotics, as the feed additives, are live microbial which can replace to avoid antibiotics utilization in animal feed. Additionally, probiotics may improve nutrients digestibility by increasing the population of beneficial microorganisms and activity of microbial enzymes [17]. Another choice could be prebiotics that are non-digestible compounds and beneficially affects intestinal useful microorganisms' growth, increasing the absorption surfaces and improve the energy and protein efficiency ratio of animals [18]. Moreover, reducing in number of pathogenic bacteria and increasing the population of beneficial microflora in the gut is reported by mixed application of probiotic and prebiotic [19]. Moreover, protein efficiency can increase due to decline in microbial proteins breakdown to N [20].

Another potential alternative for antibiotics usage is organic acids which have been previously studied in term of food conservation with reasonable achievements. Propionic, fumaric, citric, formic, lactic and butyric acids are the organic acids that were studied frequently as dietary acidifiers in animal nutrition [21]. The main antibacterial effect of organic acids is associated with disrupt normal physiology of pH-sensitive bacterial strains (such as *E. coli, Salmonella* spp., *C. perfringenes, Listeria monocytogenes* and *Campylobacter* spp), which decrease bacterial viability [22]. The positive effects of organic acids and their derivatives on the intestinal microbiota and morphology in chicken diet can increase immunity responses, however, studies have not completely support immunological function, and more studies are needed.

Sustainable Strategies to Improve Nutrients Bioavailability in Poultry Nutrition

The sustainable nutrition approaches mostly focus on investigate and development practices by which diet nutrients utilize more efficiently, as well as nutritional requirements estimate more accurately for poultry nutrition. Both above-mentioned strategies are practical especially to reduce N and P losses. The ideal protein concept, for example, formulate the dietary protein/amino acid amount based on the precise nutritional requirements of animals. The feed supplements, such as probiotics, prebiotics, organic acids and enzymes can be applied to improve the feed digestibility and absorption of nutrients or to adjust the gut microflora, result in higher bioavailability of nutrients by poultry. In addition, adding components like enzymes to poultry diet can increase raw materials diversity, which lead to utilize local potentials, decrease feed cost, etc. For instance, wheat and barley which are able to supply vastly and relatively lower cost, are specified by particularly great levels of NSPs, which affect the mono-gastric animals' digestive activity and total feed nutritional value adversely. The poultry feed supplement of mono- and multi-enzyme preparations can improve nutrient digestibility and utilizations [23].

About 50-80% of total P content of cereal grains and oilseed meals is naturally bound in the forms of phytate and phytin (unavailable form of P for non-ruminants due to lack of sufficient endogenous phytase enzyme) [24]. Hence, inorganic P resources are applied in feed formulation of mono-gastric animals, usually in excess, resulting in excess faecal P excretion and environmental impacts. For instance, poultry and swine are responsible for 36% of the total P output by manure [25]. In order to reduce P excretion, feed P contents should be estimated to the accurate requirements of animals, and apply practices to increase P bioavailability in poultry production. Although NRC (1994) recommendations of dietary P are considered to formulate poultry diets, recently performed studies have been revealed that some assessments are considered a few out-of date research publications or unsupported models [23].

The next strategy to decrease P in poultry excreta is adding phytase enzyme as a feed supplement. Phytase enzyme can breaks down phytic acid and releases P and other components. Waldroup et al. [26] reported approximately 50% enhancing in bioavailability of dietary phytate, and faecal P output was reduced by 28-47% when phytase added to broiler feed. In addition, improving P bioavailability of poultry feed can reduce poultry feed industry dependency to the unsustainable inorganic P resources, while dietary added phytase provides an opportunity for consuming from renewable P resources. Since the phytic acid forms chelates with minerals (such as calcium, zinc, iron and magnesium) which lead to reduction in bioavailability of minerals of feed, it makes another necessity of applying poultry feed supplemented by phytase enzyme. Moreover, feed ingredient is a significant factor to determine P excretion, whereas P availability is lower in dietary corn as it is for wheat or barley (23% versus 35% and 43% respectively) [27].

Another sustainable nutritional strategic approach is phase feeding, in which nutritional requirements are estimated according to growing stage. Because physiological stage that varies due to different age can affect nutritional demands. Therefore, formulate poultry diet by considering the age will be beneficial to avoid nutrients waste. For instance, increase of a single phase feeding to a two-phase, lead to decrease N excretion by 10% in livestock [28]. Preformed studies showed the benefits of multi-phase-feeding in poultry production. According to Pope et al. [29], decreasing dietary lysine, threonine, and sulphur amino acid contents, as the finisher broilers ration, resulted in reducing crude protein intake and N excretion and improved growth performance without no negative effect on the carcass traits. According to Ling et al. [30], dividing broiler chickens nutrition to four feeding period including starter (0-18d), grower (18-32d), finisher (32-42d), and withdrawal (42-49d) led

to reducing dietary non-phytin phosphorus (NPP) requirement by 5%, 15% and 40% for grower, finisher and withdrawal diets respectively, when they compared with average commercial levels without no adverse effect on bone strength, bone mineralization and performance.

Conclusion

Since, poultry production can have certain sustainably privileges comparing with other animal protein production system, many scientific studies have been done to find the sustainable methods in different aspects of industrial poultry rearing such as nutrition and management. Poultry nutrition as a major issue is obviously associated with impacts that cause the human health and environmental hazards. Fortunately, the numerous scientifically approaches have been explored during last decades that respond sustainably against hazardous impacts. The most of solutions are application of the natural origin feed supplementations such as organic acids, certain enzymes, probiotics and prebiotics that their benefits in poultry nutrition and producing healthy products were well studied scientifically.

To conclude, industrial poultry production as a significant member of nowadays food supply chain should be adapted to the sustainable feeding strategies and practices by which can be able to protect the global food security with the least unwilling impacts.

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P21 Molecular Characterization and Phylogenetic Analysis of Chicken-Derived Adenoviruses

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Abstract

In recent years, there has been an increase in chicken-origin Adenovirus infections (FAdV) and economic losses due to the disease have also been increasing. In this study, molecular characterization and phylogenetic analysis of chicken origin Adenoviruses were aimed. In the study, liver samples taken from 1488 broiler flocks clinically suspected for FAdV between 2020 and 2022 were analyzed by molecular PCR. Of 1488 broiler flocks with clinical suspicion of FAdV, 579 (38%) were found to be positive for FAdV. 123 samples selected from the samples found to be positive were typed using the Sanger sequencing method. Sequences obtained as a result of typing were created with a phylogenetic tree model based on genetic distances from reference sequences obtained from the NCBI GenBank database and showing neighborhood relationships. While 92.7% of the 123 samples sequenced were found to belong to the FAdV-8b serotype, 7.3% were found to be FAdV-11. According to the findings, it was determined that FAdV-8b was responsible for most of the broiler flocks showing clinical symptoms. These results revealed the necessity of taking necessary precautions for monitoring and control of FAdV infection in broiler flocks.

Keywords: Adenovirus, broiler, molecular characterization, phylogenetic analysis

P22 Molecular Diagnosis of Reovirus Infection in Broiler Flocks

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Abstract

Molecular diagnosis of Reovirus infections in broiler flocks was aimed in this study. In the study, samples obtained from the leg joints (femorotibial) of 5 different animals from 37 broiler flocks at different ages were used. Nucleic acid extraction of the taken joint samples was performed robotically and nested PCR was performed targeting the Reovirus σC sequence. Twenty-two (59.4%) of 37 flocks analyzed were found to be positive for reovirus infections of poultry origin. According to the findings, it was also noted that Reovirus infections were quite common in broiler houses and the rate of positivity increased with age.

Keywords: Broiler, PCR, Reovirus

P23 The Using of Intelligent Indicator Labels in Packaging of Chicken Breast Fillets

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Abstract

Recently, intelligent labels that have been seen as a part of innovative packaging system in the meat industry have come into use. Thanks to these labels, consumers can get information about the meat quality (fresh or spoilage) by looking at color changes in the labels. Intelligent labels are objects that can be attached to various parts of packaging material in the form of paper or film. In this study, the five different indicator paper-based natural and synthetic dyes were developed and attached to the inside of the packaging materials using double-sided tape, and also the color changes that occurred in the labels during storage were investigated. Although the indicator labels-based synthetic dye observed color changes in a shorter time compared to the indicator labels-based natural dye, the number of 3, 4, and 5 samples were determined obvious color changes after storage of 10th days. Moreover, physicochemical and microbiological properties in the modified atmosphere packaging (%30 CO_2 and %70 N_2) samples were investigated during storage (4ºC, 20 days). The results showed that the pH value, TBARS value, carbonyl content, total psychrophilic aerobic, total coliform, and total yeast-mold counts in the chicken breast fillets were significantly increased during storage periods (4ºC, 20 days).

Keywords: chicken breast fillet, smart packaging, shelf life, natural and synthetic dyes

P[24] Thermal Manipulation of Broiler Embryos: Effects on Body Weight, Meat Quality and Incidence of Myopathies

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Abstract

The present study aimed to evaluate the effect of cyclic high incubation temperature on body weight, the incidence of myopathies, and meat quality in broilers. A total of 392 eggs were obtained from Cobb broilers and divided into 2 groups, randomly. The first group was incubated at 37.8°C between embryonic days (ED) 0 and 18 as a control group and the second group was exposed to heat treatment (HT) for 6 h between ED 10 and 14 where the temperature was 38.8°C. After hatching, chicks from both groups were reared in similar conditions. All chickens from each group were weighed at 21 and 42 days. On d 42, all broilers were slaughtered and white striping (WS), wooden breast (WB) and spaghetti meat (SM) were scored. Pectoralis major muscles were sampled to analyze nutrient content and meat quality. At 21 days body weight was found similar in both groups however at 42 d HT broilers were heavier than the control. HT didn't affect the incidence of WS, WB and SM. Breast muscle pH_{15} was higher in the control group than HT; however, pH_{24}, L^*, a^*, b^*, drip loss and cooking loss were similar in both groups. HT had no impact on dry matter, ash, or protein content whereas fat content was lower in HT groups. The results suggested that ~~although~~ cyclic high incubation temperature had no effect on muscle degeneration, however, it improved the body weight of broilers and reduced breast meat fat content.

Introduction

Between 2000 and 2019, global meat consumption increased from 29.5 kg per capita to 34 kg, while poultry meat shared 41% of all meat consumption [1]. In response to consumer demands, broiler breeders have focused on achieving a heavier slaughter weight in a short growing period by genetic selection. The genetic selection also increased breast meat yield, however, as an unexpected result of the progress achieved in live weight and breast weight by genetic selection, it caused changes in the histological and metabolic properties of the breast muscle; e.g. a decrease in muscle glycogen reserve and an increase in muscle fiber diameter and muscle myopathies [2]. While the incidence of myopathies has increased rapidly during the last years, the most common myopathies are white striping (WS), wooden breast (WB), and spaghetti meat (SM) [3, 4, 5]. In broilers, 5-53.2% and 41-72 % of the breast muscles suffer from wooden breast and white striping [6, 7, 8] respectively, and 20% of spaghetti meat [4]. Along with the altered appearance, myopathies have an impact on meat quality resulting in significant changes in nutrient composition in the breast muscle, especially protein and fat content [9, 10].

Environmental conditions during embryonic development are critical for muscle development. Incubation temperature, which is 37.5-37.8^{0}C, may have an effect on muscle development affecting the number and structure of the muscle fiber, which results in a long-lasting effect on postnatal muscle growth [11, 12]. It was shown that thermal manipulation during the mid-and late-term of embryogenesis can have long-term simulative effects on muscle growth by promoting myogenic cell proliferation and differentiation [13]. A 39.5^{0}C for 3 or 6 h/day between embryonic days (ED) 16 and 18 was shown that affected myofiber hypertrophy and muscle development through the proliferation and differentiation of myogenic cells [14]. Al-Zghoul et al. [15] reported that 39.5^{0}C for 12h/day between ED7 and 16 improved muscle hypertrophy, which led to heavier body weight at post-hatch. Incubation temperature of 39.5°C from ED14 to 18 for 12 h per day also lowered the severity of myopathies without negatively impacting meat quality [16]. Although many studies have focused on effect of incubation temperature on broiler performance and muscle properties, it is difficult to get a consensus. Therefore, the present study was conducted to investigate the effect of a cyclic higher incubation temperature from ED 10 to 14 on post-hatch growth performance, meat quality, and incidence of WS, WB, and SM myopathies at slaughter age.

Material and Methods

A total of 392 eggs obtained from a 52-week-old Cobb broiler breeder flock were placed within 2 incubators (196 eggs

capacity each). One of these incubators was kept as the control group (C) where eggs were exposed to 37.8°C and 60% humidity between ED 0 and 18 and the eggs in the second incubator were exposed to heat treatment (HT) for 6 h between ED 10 and 14 where the temperature was 38.8°C with 60% humidity. On day 18 of incubation, the eggs were transferred to hatching trays and kept at 36.8°C and 70% humidity until hatching.

On the day of the hatch, a total of 76 chicks from each incubation temperature were weighed and distributed into four-floor pens. Rearing conditions were provided according to Cobb 500 Broiler Management Guide [17]. Feed and water were provided ad libitum. All chicks from each group were weighted at 21 and 42 days. On d 42, all broilers were slaughtered and WS, WB, and SM breasts were visually scored according to references [7, 18, 19, respectively]. A total of 12 pectoralis major muscle/incubation group were sampled to analyze nutrient content and meat quality. The pH was measured at 15 minutes (pH_{15}) and 24 h (pH_{24}) after slaughter using a portable pH meter. Lightness (L^*), redness (a^*), and yellowness (b^*) were determined at 24 h after slaughtering using a Minolta Colorimeter (Minolta CR300). Drip loss and cooking loss were determined according to Brannan et al. [20]. Breast meat samples were kept at -20^{0}C until nutrient content analysis. Breast protein content was evaluated by the Kjeldahl method, and CP was calculated as N × 6.25. Lipid content was determined by AOAC (1990). Protein and fat content were expressed as percentage of total dry matter. Data were analyzed with using General Linear Model (GLM) procedures of JMP 5.0.1software. T- Test model were applied when differences were significant. Chi-square test) $\chi 2$ (was applied for analyzing muscle myopathies.

Results and Discussion

Piestun et al. [14] reported that HT from ED 16 to 18 (39.5◦C for 3 or 6 h/day) improved the body weight of broilers at day 35 in comparison with the control group. In contrast, Al-Zghoul et al. [15] showed that 39°C between ED 12 and 18 for 9 h/day had no significant effect on body weight at 21 or 35 days, however, the same higher temperature when applied at 12 or 18 h/day led to heavier broiler at day 35. In the present study, the body weights of birds from two incubation temperatures were similar at 21d (Figure 1). At slaughter age, HT broilers were heavier than those C. This result suggested that HT applied in this study improved the slaughter weight of birds.

Thermal manipulation during embryogenesis may help to decrease the incidence of myopathies [16, 20]. However, in our study, the effect of temperature on the incidence of myopathies was not significant (Table 1). This may be related to 1) the HT applied in our study, which was shorter than the others and 2) the early slaughter age of the birds, since the incidence of myopathies increased with the slaughter age.

After slaughtering, the amount of lactic acid in breast muscle increases due to postmortem glycolysis, which leads to a decline in muscle pH [21]. Since pH decline is related to protein denaturation, it affects meat quality by changing the color and water-holding capacity [22, 23]. The HT broilers had a lower pH_{15} compared to C broilers (6.41 vs. 6.53) which was considered as approached significant (P=0.058). This result did not agree with the findings of Janisch et al. [24] who reported a higher pH_{20} at slaughter age (35 days) in the HT group (38.8^{0}C between ED 10-13) in comparison to the C. However, pH_{24} was similar between treatment groups. No significant differences were found between C and HT groups for either color profile or water-holding capacity. Similarly, Werner et al. [25] and Krischek et al. [26] showed that 38.5^{0}C between ED7 and 10 or ED 9 and 12 did not affect meat quality. These results indicated that higher incubation temperature (38.8^{0}Cfor 6h/day) between ED 10- 14 had no negative effect on meat quality at slaughter age.

The other important meat quality parameter is nutrient composition. In our experiment, dry matter, ash and protein contents of breast muscles were similar in C and HT broilers. However fat content was higher in the breast meat of the C broilers in comparison with the HT group (Figure 2).

In conclusion, the present study showed that although HT did not have an impact on the incidence of muscle degenerations, it positively affected body weight at slaughter age and modified breast fat content of broilers.

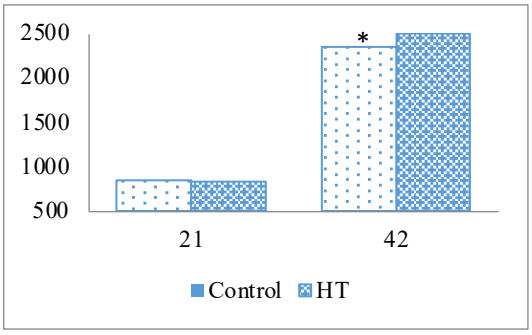

Figure 1. Effect of treatments on live weight of broilers at 21 and 42 days

Table 1. The effect of incubation temperature on muscle degeneration (%) at slaughter age

	WS			WB		SP	
	0	1	2	0	1	0	1
Control	53.19	38.30	8.51	97.87	2.13	87.23	12.77
HT	56.45	40.32	3.23	96.77	3.23	90.32	9.68
Probability		0.488			0.729		0.610

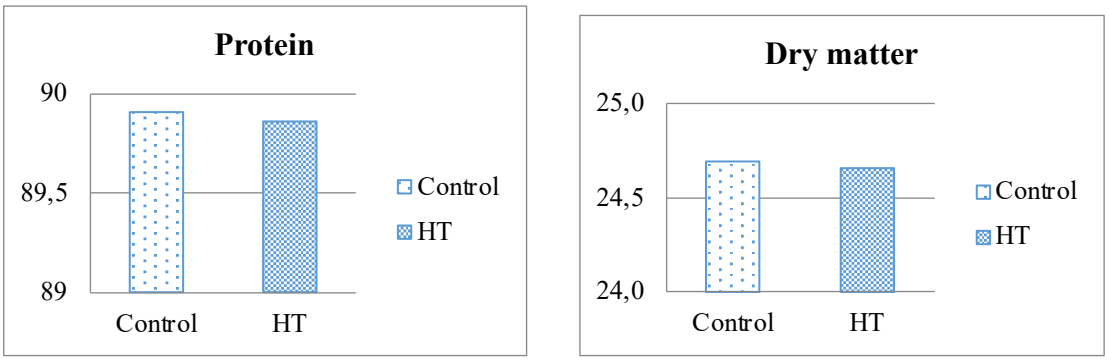

Figure 2. Mean percentage of dry matter, ash, protein, and fat content by incubation temperature at slaughter age

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P25 Pen Enrichment on Tibia Characteristics of Fast- and Slower-Growing Broilers

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Abstract

This study describes effects of a combination of various pen enrichments consisting of ramps, platforms, perches, large distance between feed and water and provision of live Black Soldier fly larvae in the moss-peat dust bathing area on tibia morphological, biophysical and mechanical characteristics of fast- and slower-growing broilers. The experiment was set up as a 2 x 2 factorial design with two broiler strains (Ross 308 and Hubbard JA 757) and two pen types (enriched and non-enriched). A total of 840 male broiler chickens (7 pens per treatment and 30 chickens per pen) were used at similar body weights. Chickens in non-enriched pens had a lower tibia osseous volume (Δ=1.8 cm^3, P=0.003), total volume (Δ=1.4 cm^3, P=0.03) and volume fraction (Δ=0.02%, P=0.002) than chickens in enriched pens, suggesting that pen enrichment particularly affects ossification and mineralization mechanisms. Slower-growing broilers had a higher tibia osseous volume (Δ=5.9 cm^3, P<0.001), total volume (Δ=5.4 cm^3, P<0.001), volume fraction (Δ=0.05%, P<0.001), mineral content (Δ=0.7 g, P=0.02) and mineral density (Δ=0.05 g/cm^3, P<0.001) than fast-growing broilers at the same body weight. It can be concluded that pen enrichment positively affected tibia biophysical characteristics in both fast and slower-growing chickens, while no effect was found on tibia morphological and mechanical characteristics. This suggests that pen enrichment can stimulate pathways involved in bone ossification and mineralization, rather than morphological and physical bone characteristics.

Keywords: pen enrichment, broiler, fast-growing, slow-growing, tibia

Introduction

Broiler chickens have undergone radical phenotypic and genotypic changes as a result of selection for a rapid growth rate in the last century (Zuidhof et al., 2014; Tallentire et al., 2016; Bessei, 2018). Alongside the advantages of this rapid growth, it has also led to several problems, including impaired leg health and bone deformations, which might result in increased pain, poor welfare, lack of locomotion, higher mortality, lower slaughter revenues and significant financial losses (Kestin et al., 1999; Mench, 2004; Bradshaw et al., 2002; Bessei, 2006; Knowles et al., 2008; Gocsik et al., 2017). One of the strategies to promote leg health of broilers might be to stimulate activity and locomotion by pen enrichment (Reiter and Bessei, 2009; Blatchford et al., 2012; Ohara et al., 2015; Pedersen et al., 2019). Several behaviour-related studies showed that broiler chickens spend approximately 80% of their lifespan passively (e.g., lying, sitting and resting) (Weeks et al., 2000; Reiter and Bessei, 2009; Zuidhof et al., 2014). Together with a fast growth rate, passive behaviours may impair bone development, which can result in suboptimal leg health and lameness (Balog et al., 1997; Bradshaw et al., 2002; Reiter and Bessei, 2009).

Another potential strategy to promote leg health and welfare is to reduce growth rate of broilers. It has been found that slower-growing broilers spent more time on perches and platforms (Bokkers and Koene, 2004; Wallenbeck et al., 2016), showed better locomotion (Weeks et al., 2000; Cornetto et al., 2001; Reiter and Kutritz, 2001; Bokkers et al., 2004) and had less leg problems than fast-growing broilers.

The aim of this study was to investigate effects of a combination of various pen enrichments (ramps, platforms, perches, large distance between feed and water and provision of live Black Soldier fly larvae in the moss-peat dust bathing area) on tibia morphological, biophysical and mechanical characteristics (at similar body weights) of fast- (Ross 308) and slower-growing (Hubbard JA 757) male broilers.

Materials and Methods

The experiment was setup as a 2 x 2 factorial arrangement with two strains of broiler chickens (Ross 308: fast-growing or Hubbard JA 757: slower-growing) and two different levels of pen enrichment (enriched or non-enriched). Enriched pens contained two wooden platforms (one at each long side of the pen), two angular (11.5°) wooden ramps, a dust bathing area with peat moss, two vertical wooden barrier perches, adjustable in height, a maximum distance (3 m) between feeders and

drinkers and provision of live Black Soldier fly larvae (BSFL) in the dust bathing area (please see details in Güz et al., 2022). Non-enriched pens included feed and water (at 1 m distance) and one single long perch (300 x 4 cm, not adjustable in height). Illustrations of the enriched and non-enriched pens are provided in Figure 1. Fast-growing broiler chickens were reared till day 38 of age, whereas slower-growing broiler chickens were reared till day 49 of age. The experiment was conducted at the research accommodation of Wageningen Bioveterinary Research (Lelystad, The Netherlands). All procedures were approved by the Central Commission on Animal Experiments (The Hague, The Netherlands). A total of 420 fast-growing (Ross 308) and 420 slower-growing (Hubbard JA 757) day-old male broiler chickens were randomly allocated to 28 pens (7 pens per treatment, 30 male broiler chickens per pen). At a body weight of approximately 1,400 gram (day 29 and 38 of age for fast and slower-growing chickens, respectively) and approximately 2,200 gram (day 38 and 49 of age for fast and slower-growing chickens, respectively) 3 chickens per pen were slaughtered and the tibia bones of the right legs were collected. Tibia weight, proximal length, lateral cortex thickness, osseous volume, pore volume, total volume, volume fraction, mineral content and mineral density were analysed on each tibia, using a 3D X-ray microfocus CT scanner (described in Güz et al., 2019, 2021, 2022). The same tibias were then subjected to a three-point bending test. Ultimate strength, yield strength, stiffness and energy to fracture were measured (Güz et al., 2019, 2021, 2022). Tibia characteristics at the two body weight classes were subjected to general linear mixed model analysis, using PROC MIXED in SAS (Version 9.4, SAS Institute Inc., Cary, NC, US).

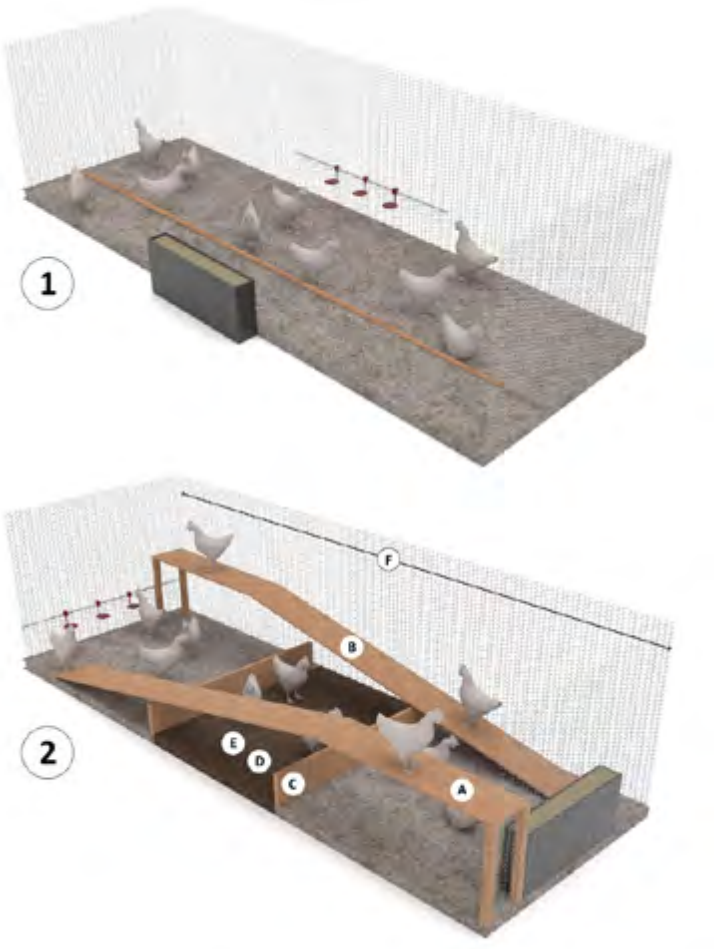

Figure 1. Illustrations of non-enriched (1) and enriched (2) pens. (1) Non-enriched pens contained a short distance (1 m) between feeders and drinkers placed on opposite long walls, had one non-adjustable perch and the pen was covered with wood shavings as a bedding material. (2) Enriched pens contained two wooden platforms (A; 100 x 20 x 40 cm, one at each long side of the pen), two wooden ramps (B; 200 x 20 cm, angle of 11.5°), two vertical wooden barrier perches (C; 100 x 4 cm, adjustable in height from 4-16 cm with steps of 4 cm at days 7, 14 and 21), one dust bathing area (D; 100 x 100 cm) with peat moss and provision of live Black Soldier fly larvae in the dust bathing area (E) and a large distance (3 m) between feeders and drinkers placed on opposite short walls (F).

Results

Body Weight of 1,400 g **:** No interaction effects between pen enrichment and strain were found on tibia morphological characteristics and neither pen enrichment effects were found. Slower-growing broilers had a higher tibia osseous volume (Δ=6.4 cm^3, P<0.001), total volume (Δ=6.6 cm^3, P<0.001), volume fraction (Δ=0.04 %, P<0.001), mineral content (Δ=1.1 g, P<0.001), ultimate strength (Δ=21.7 N, P<0.001), yield strength (Δ=21.0 N, P<0.001), stiffness (Δ=20.6 N/mm, P<0.001) and energy to fracture (Δ=21.9 N-mm, P<0.001) than fast-growing broilers.

Body Weight of 2,600 g : An interaction between pen enrichment and strain was found for tibia pore volume. The enriched housed slower-growing chickens had a lower tibia pore volume compared to other three groups (Δ=1.0 cm^3 on average; P=0.02). Slower-growing broilers had a higher tibia weight (Δ=0.81 g, P=0.02), proximal length (Δ=0.63 cm, P=0.008), osseous volume (Δ=5.9 cm^3, P<0.001), total volume (Δ=5.4 cm^3, P<0.001), volume fraction (Δ=0.05 %, P<0.001), mineral

content (Δ=0.7 g, P=0.02), mineral density (Δ=0.05 g/cm^3, P<0.001), ultimate strength (Δ=19.4 N, P<0.001), yield strength (Δ=17.8 N, P<0.001), stiffness (Δ=21.7 N/mm, P<0.001) and energy to fracture (Δ=20.9 N-mm, P<0.001) than fast-growing broilers. Chickens housed in non-enriched pens had a lower tibia osseous volume (Δ=1.8 cm^3, P=0.003), total volume (Δ=1.4 cm^3, P=0.03) and volume fraction (Δ=0.02 %, P=0.002) than chickens in enriched pens.

Dıscussion

Results of this study showed that tibia osseous volume, total volume and volume fraction of both fast- and slower-growing broiler chickens and tibia pore volume of slower-growing chickens were only positively affected by pen enrichment, while most of the other tibia characteristics were slightly higher, but not significant. These findings are in agreement with previous studies, indicating the stimulating effects of pen enrichment on bone characteristics (Sørensen et al., 2000; Toscano et al., 2013; Pedersen et al., 2020). Bone mineral deposition was the most stimulated physiological charecteristic by pen enrichment, whereas tibia morphological and mechanical characteristics were not significantly affected. It can be hypothesized that stimulating activity due to pen enrichment particularly affects physiological pathways involved in ossification and mineralization, rather than affecting morphological and physical tibia characteristics. Regarding the strain, almost all tibia characteristics in both body weight classes were higher in slower-growing chickens than in fast-growing chickens. These findings are in line with previous studies, indicating that slower-growing chickens demonstrate better bone characteristics at all ages compared to fast-growing chickens (Thorp and Waddington, 1997; Williams et al., 2000; Torres and Corver, 2018). Fast-growing chickens have more porous and less mineralized leg bones than slower-growing broiler chickens, which together with a higher body weight gain results in a higher risk of lameness (Sullivian, 1994; Williams et al., 2000, 2004; Shim et al., 2012; Torres and Corver, 2018).

Conclusion

In both slower and fast-growing chickens, tibia biophysical characteristics, in terms of bone mineral depostion, were positively influenced by comprehensive pen enrichment, while tibia morphological and mechanical characteristics were not affected, suggesting that pen enrichment particularly affects physiological mechanisms related to ossification and mineralization. Slower-growing chickens showed better tibia characteristics than fast-growing chickens.

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P[26] How Do We Use Molecular Information to Control Pandemic Poultry Viruses?

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Abstract

Pandemic respiratory viruses of poultry have caused significant economic losses to the poultry industry since the 1930s. Molecular and genetic techniques are widely used in the diagnosis and control of these infections. Knowing the changes in the genetic and antigenic properties of pandemic viruses over time can be really important for recognizing viruses that cause pandemics in humans, such as SARS-CoV-2 and Influenza virus. The use of these techniques plays a vital role in preventing faulty results and possible financial losses due to limited findings obtained from conventional laboratory tests. In the light of this information, the aim of this review is to provide an up-to-date assessment of the diagnosis and prevention of major respiratory viruses in poultry and a general and field-oriented scientific perspective that may be useful to the industry. Briefly, proper vaccine selection and the control of the immunization mostly depend on the actual molecular detection and genetic characterization approaches. In this context, current approaches to diagnosis and vaccination applications developed using molecular methods based on Avian Coronavirus Infectious Bronchitis Virus, Avian Paramyxovirus-1, and Avian Influenza Virus, which cause pandemics, are discussed and solutions for effective struggle are presented.

P27 Molecular Identification, Biotyping and Determination of Antibiotic Resistance Profiles of H2S-Negative *Salmonella* Isolates in Turkiye

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Abstract

This study aimed to identify H_2S negative *Salmonella* spp. isolates of back yard chicken origin by using molecular methods, to determine their antibiotic resistance profiles and to find the biochemical differences and In the scope of this study, one gram of samples was taken from the livers, spleens and intestines of 20 back yard chicken with suspected Salmonella infection. These samples were planted directly into blood agar and MacConkey agar and inoculated into Rappaport Vassiliadis broth. Afterwards, following the isolation in differential media, suspicious colonies were transferred onto Triple Sugar Iron (TSI) agar and 12 H_2S-negative isolates were identified using Dulcitol and Ortnithine biochemical tests as *S.* Gallinarum. In order to confirm the the isolates by PCR, primers containing Salmonella type specific gene regions (16S rDNA and invA) and primers specific to enterotoxin gene(stn) responsible of Salmonella enterotoxicity were used. All 12 *S.* Gallinarum isolates were found to be positive for these three genes. 16S rDNA PCR products were sent to sequence analysis to find phylogenetic relations of the isolates. In addition, Kirby-Bauer disk diffusion test was performed in order to determine the antibiotic resistance profiles of the *S.* Gallinarum isolates. All *S.* Gallinarum isolates were determined to be resistant against 4 or more antibiotic groups and mutiple drug resistance (MDR) ratio is 100%. Higher resistance rates in the isolates were observed against the antibiotics NA(100%)/E(100%)/KZ(100%)/FOX(100%)/AMP(100%) /CAZ(83.3%)/CIP(75%). The results show that back yard chicken as a source of *S.*Gallinarum isolates having high level of antimicrobial resistance that will be infectious (cojugative or plasmid-dependent resistance) for zoonotic other Salmonella serotypes with a risk for public health.

Keywords: Salmonella, H_2S Negative, Antibiogram, PCR

P[28] Healthy Chicks from Hatchery to House with *In Ovo* Feeding

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Abstract

Poultry products are healthy and cheap protein sources that can be produced in a short time and have a very important place in human nutrition. The poultry sector, which started in the 1930s in our country and continues to progress rapidly until today, has surpassed red meat production in terms of white meat production and consumption figures and has become an indispensable animal food in human nutrition, especially in meeting protein needs. Among the reasons for the rapid development of white meat production are the genetic capacity of the chicken, managerial improvements and feeding. It is a known fact that feeding has a significant effect on the development, performance, immune system and gut health of broiler chickens. In current scientific platforms, discussions on early feeding methods other than classical feeding methods have been started and scientific studies dealing with this issue have been emphasized. One of the remarkable early feeding methods is the in ovo feeding method. With the in ovo feeding method, the nutritional and feed additives needed by the embryo are supplemented, stimulating the development of the digestive system and immune system in the embryonic period. In this way, it is aimed to start breeding with a strong chick that is ready for the digestion and absorption of nutrients, resistant to infections. In this review, the development of the in ovo technique, the transition process to in ovo feeding and related studies, the effects of in ovo feeding on intestinal development and the immune system are mentioned.

P29 Effects of Selenium in Heat Stressed Poultry Diets on Performance, Oxidative Metabolism and Immune System

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Abstract

Heat stress is an important environmental factor that negatively affects poultry performance parameters such as feed consumption, growth, egg production and metabolism, and consequently causes great economic losses in the poultry industry. Combating the negative effects of heat stress on poultry, many methods such as management (house design, feeding time, etc.) and feeding strategies (vitamin-mineral use in the ration, etc.) have been applied, but recently, studies on the use of Selenium in the diet have been increasing. Selenium takes charge in various metabolic events in the body and also the structure of various enzymes. In studies with Se in the literature, it has been reported that Se has a positive effect on performance, antioxidant parameters, immune system and some other parameters in poultry. In this review, some effects of Se application in the diets on heat-stressed poultry are mentioned.

P30 Antimicrobial Resistance in the Poultry Production Chain: Monitoring Resistance and The Role of Disinfectants in The Selection of Resistant Microorganisms

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Abstract

Infections caused by bacteria resistant to antimicrobial agents constitute one of the most important public health problems all over the world. As a result of some factors such as incorrect drug use, especially pathogenic bacteria develop resistance to antimicrobials through different biological pathways. When humans and animals are sick with the pathogens, it is not possible to cure the infections that are expected to be cured as a result of the use of antibiotics under normal conditions, and the desired results cannot be obtained in different combination and/or high-dose antibiotic treatments. This is a factor that significantly increases the incidence of morbidity and mortality. As a result of this, the use of existing antibiotics is increasing, and it brings an economic burden to the society as new agents are needed. According to the projections of the World Health Organization, it is thought that approximately 10 million people will die in 2050 due to antibiotic-resistant microorganisms. This situation, which has an increasing importance day by day, it is of great importance for human, animal, and environmental health to take urgent measures not only in human medicine but also in veterinary medicine and animal breeding.

P31 Quality Increase in Poultry Meat with the Use of Active and Intelligent Packaging Technology

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Abstract

Poultry meat; is an important food for a healthy and balanced diet with its high quality protein, low fat and rich vitamin-mineral content. The measures taken in terms of food safety and public health during the period from the farm stage to consumption are of great importance for both the producer and the consumer. Active and intelligent packaging technologies contribute to issues such as increasing food safety, protecting public health, extending shelf life, reducing food waste and increasing customer satisfaction. In addition, the quality increased by the use of these technologies in poultry meat and products provides economic benefits. In this context, the important benefits of using active and smart packaging in poultry meat and products have been tried to be expressed.

P[32] Algies in Early Feeding of Broilers as Sustainable Raw Material

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Abstract

The increasing world population increases the need for easy, accessible and cheap poultry meat production day by day. For this reason, every development that can contribute to the poultry meat production potential is very important in order to meet the demand. “Pre-hatch” and “post-hatch feeding practices, which we call the early period, seem to be able to contribute to this development by accelerating the development of the embryo and chick digestive system, and by producing chickens with higher live weights in slaughter. Inovo feeding and pre-starter feeds are feeding applications that serve this purpose. In these feeding programs, which are used as modern feeding practices today, enrichment in terms of nutrients can be provided as well as feed additives. Recently, thanks to its rich nutrient profile and bioactive components, macroalgae have also become the focus of attention in animal nutrition. Macroalgae have found a wide range of use in animal nutrition studies due to their easy production, being economical, rich nutrient content, containing bioactive components and being rich in pigments and having high antioxidant, antibacterial, anti-inflammatory and antiviral effects. In studies, it has been determined that macroalgae can be used as a raw material due to its nutrient profile, can support live weight gain, and contain active ingredients that improve meat quality. In this review, the advantages, disadvantages and the use of macroalgae as a different raw material source for sustainable aquaculture will be discussed in detail.

P33 Molecular Characterization of Infectious Bursal Disease Virus

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Abstract

Infectious bursal disease is an important disease which causes immunosuppression and economic losses in poultry. In this study, the aim was to conduct molecular characterisation and phylogenetic analysis of infectious bursal disease viruses detected from bursae of Fabricius which were collected from different types of poultry flocks. Twenty-five bursae of Fabricius collected from chickens suspected of having infectious bursal disease (IBD) in different types of poultry flocks were examined using molecular techniques. Of twenty-five samples, seventeen of them were found positive for IBDV. Positive samples were typed by Sanger sequencing. The sequences of positive samples obtained from Sanger sequencing and the sequences of reference strains were compared using BLAST and phylogenetic trees of the aligned sequences were generated using the neighbour joining method in the same software. All of the isolated viruses except one were detected to be vaccine strains as a result of the analyses of both VP1 and VP2 genes. As a result of this study, IBD viruses detected as classical strains were found to be different strains according to the analyses of VP1 and VP2 genes even though they all are in the same genogroup. Therefore, it is shown that analysing both VP1 and VP2 genes is important when conducting phylogenetic analysis of IBD viruses in chickens.

Keywords: Infectious bursal disease, molecular characterisation, phylogenetic analysis

P[34] Isolation and Characterization of *Salmonella Infantis* Phages From Chicken Feces and Environmental Samples

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Abstract

In this study, it was aimed to isolate the bacteriophages of *S.* Infantis, the most isolated *Salmonella* serotype of poultry in Turkey, to determine the effect and lifespan in the water, litter and feed and to detect the host spectrum and the storage time of these phages. In this study, the routine test dilutions, lytic spectra and lytic profiles of 38 *S.* Infantis phages isolated from 50 stool-litter and 50 wastewater samples were determined and the selected phages were genotyped by RAPD-PCR. Phage-bacterial dynamics of phages with the highest litic spectrum (SF-In7, SF-In20) selected among the different litic profiles and RAPD homology levels were investigated. As a result of the study, SF-In7 and SF-In20 phages can be used as biocontrol agents to reduce the *S.* Infantis contamination, and can be stored easily for a longtime period before application in environments such as field, poultry house, slaughterhouse due to its large storage temperature and long life span.

P35 Effect of Vitamin E on Meat Quality in Broiler Chickens

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Abstract

For the production of poultry meat, the necessary nutrients should be given to the animals to ensure that they are fed adequately and in a balanced way. Vitamin E added to the ration is an important antioxidant for poultry. It is important for both metabolism and meat quality. Vitamin E preserves the integrity of the long-chain polyunsaturated fatty acids in cell membranes and maintains their bioactivity.Oxidative stress results from an imbalance between prooxidants and antioxidants at the cellular or individual level. Oxidative stress is a factor that inhibits growth and development in poultry. Vitamin E is a beneficial substance that counteracts oxidative stress. It is of great importance that vitamin E, which increases meat quality, shelf life, reduces lipid oxidation, and has an important place in the strengthen immune system, should be present in the diet.

Keywords: Meat quality, oxidative stress, vitamin E, broiler

P[36] Evaluation of Poultry Offal Liver in Nugget Form

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Abstract

The poultry industry, the largest producer of animal protein, is the focus of mixed diet consumers alongside the livestock industry in general. Poultry meat's variable but generally moderate energy content and high-quality, highly digestible proteins make it prominent in healthy nutrition. Unsaturated lipids, which are mainly found in the skin and can be easily separated during consumption, B group vitamins such as thiamine, vitamin B6 and pantothenic acid, minerals such as iron, zinc and copper make poultry meat a valuable food in terms of nutrition. Epidemiological studies conducted worldwide in a wide variety of populations with different food preferences and dietary habits provide important information about the relationship between poultry meat consumption in a balanced diet and health.

Communities have better understood the importance of reaching agriculture and food in the face of the food needs of the ever-increasing population all over the world, the problems caused by the pandemic conditions, and the negative effects of climate change on agriculture. For the last 50 years, the poultry meat industry has been working rapidly to develop environmentally friendly and sustainable production practices in order to provide a healthier planet. Large amounts of by-products are also produced with poultry meat production. Poultry meat production in the world in 2021 is 133.35 million tons. This production is expected to be 133.62 million tons in 2022. Approximately 90% of this belongs to the production of 119.5 million tons of chicken meat. On average, 25% of the production is not offered to the end consumer and is considered as a by-product. Chicken liver, which is one of the internal organs of chickens, is offered directly to the consumer, but a large amount of it can be found among the raw materials used in the production of different products by going to rendering. The rich protein, iron, vitamin A and vitamin B12 content of chicken liver has an important place in human nutrition, and its use in different formulations can make the product a functional food. Presenting it to the consumer in this functional state will contribute to the increasing need for food in the changing world order and to the circular economy model.

In this study, it was aimed to utilize the liver, which is one of the by-products of chicken meat, as a functional, healthy snack with high protein, iron and vitamin content by making liver pate, which has gastronomic properties and high nutritional and sensory properties.

Keywords: poultry industry, poultry offal, liver, nugget

P37 The Effect of Probiotic Use on Gut Microbiota and Body Weight of Broilers

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Abstract

Probiotics are frequently used to regulate gut microbiota and improve performance. In this study, it was aimed to investigate the effects of drinking water and probiotic use on gut microbiota and live weight in broilers. The study was carried out in a total of 128 Ross 308 broilers. The probiotic was used throughout the entire production period. On the 7th, 14th, 21st, 28th, 35th and 42nd days of the production period, broilers were individually weighed and body weight calculations were made. For microbiome analysis, a total of 16 greyhound feces samples were collected, 8 representing each group in the experimental and control groups. The digestive tract microbiota was determined by next-generation sequencing and metagenomic analysis. When the body weight findings were evaluated, the body weights of the experimental group given probiotics were found to be higher than the control group in all weeks of weighing. It was observed that the live weight was 52 g higher in the group given probiotics at the slaughter stage. As a result of microbiome analysis, Firmicutes, Actinobacteriota and Gastranaerophilales bacterial phyla were found to be higher in the experimental group compared to the control group, while Bacteroidales bacterial phyla were found to be lower. As a result, it was seen that the use of probiotics increased the beneficial bacterial phyla that make up the intestinal microbiota and showed a uniform distribution, and accordingly, the body weight in the experimental group was higher than in the control group.

Keywords: Body weight, broiler, gut microbiota, probiotic

P[38] Growth Performance and Nutrient Composition of Flour Wolf (tenebrio molitor) Fed on Yeast and Compost

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Abstract

In this research, the impacts and nutritional values of using various feed ingredients on the growth performance of mealworms (*Tenebrio molitor* L.) were investigated. The experiment was carried out with 1300 T. molitor larvae for 5 weeks. Mealworms were divided into 3 groups as one control (bran) and two experimentals (compost&bran and yeast &bran). Bran was included in the diet of the control groups, along with carrots as a source of water. In the yeast groups, which is one of the experimental groups, 50% bran and 50% yeast were added. In the other experimental group, compost groups, 50% bran and 50% compost were supplied and carrot was provided as a source of water. In the experiment, it was determined that the difference between the 5th week was very significant according to the values measured in the first week of mealworms whose CAs were measured at the 5th week. However, the lengths of the mealworms differed between the groups and mealworms which were fed with compost and yeast were measured longer than the other control groups. Between the groups; no difference was observed in terms of crude protein and crude ash, however, a significant difference was found between the raw oil and dry matter ratios, where the experimental groups had higher raw oil and higher dry matter than the control groups. As a result, mealworms contain high levels of protein and their nutritional values may differ according to their diet.

P39 Effects of Adding Muramidase to Broiler Feeds

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Abstract

Muramidases form a superfamily of enzymes that hydrolyze peptidoglycan (PGN) in bacterial cell walls. Accumulation of PGNs in the intestinal lumen can impair gastrointestinal functionality and performance. In this review, we examined the effects of muramidase on performance and gut health parameters when added as a feed additive to broiler diets. The results suggest that muramidase added as a feed additive to broiler feed activates nucleotide-binding oligomerization domain-containing protein 2 (NOD2) receptors by degrading PGN to muramyl dipeptide (MDP), which is present in eukaryotic cells in the intestinal lumen. In this way, muramidases have been shown to improve gut health and inhibit excessive immune system responses in the host. Digestion and absorption of nutrients are improved as amino acids are not wasted in excessive immune system reactions, and also because the ability of muramidase to hydrolyze PGNs clears the intestinal lumen of bacterial cell wall debris. In short, as a result of improved gut functionality, improvements in performance values such as feed conversion (FCR) and European Production Efficiency Factor (EPEF) were detected.

P40 Protease Enzyme in Broiler Nutrition: More Than Protein Digestion ?

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Abstract

Exogenous feed enzymes are used to improve the utilization of feed by broiler chickens. In addition to improving performance, exogenous enzymes addition to diets could reduce feed costs while maintaining broiler performance. Protease is one of the exogenous enzymes that has recently been added alone to broiler diets and there has been ongoing investigation of its effects largely on protein and amino acid digestibility. It was mostly shown that protease addition improved protein and amino acid digestibility in broilers. Yet, some researchers have suggested that protease addition may have significant effects beyond protein and amino acid digestion. Supplementation of exogenous protease may cause disruption in the feed nutrient matrix and improve the digestibility of starch and fat in broiler chickens. This increased digestibility has been demonstrated to result in improved energy utilization as well. Some researchers have also indicated that improving energy utilization may be attributed to changing digestive dynamics in addition to improved nutrient digestibility. In this respect, protease supplementation was shown to reduce heat increments and increase the net energy value of broiler diets. Increased digestibility of proteins and amino acids in the proximal intestine through increased rates of digestion due to protease supplementation is suggested to contribute to maintaining gut health. Furthermore, decreased digesta viscosity, improved intestinal morphology and expression of some genes related to gut integrity which have been reported in some studies indicate that protease addition has beneficial effects on gut health. Overall, protease addition to diets may improve fat and starch digestibility, energy utilization, and gut health in broiler chickens.

Keywords: broilers, digestibility, energy utilization, gut health, protease

P41 Recent Advances in the Use of Insect Meal as an Alternative Protein Source in Poultry Nutrition

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Abstract

Soybean meal, Sunflower seed meal and fish meal are important traditional protein sources in poultry nutrition. The decrease in protein sources due to drought, pandemic and wars is the biggest problem of the world poultry industry.

Insects are natural food for poultry. Poultry consume worms, insects and their larvae in their natural environment. On the other hand, insects can reduce pollution by consuming animal manure and food scraps, while at the same time converting waste into proteins, it reduces total nitrogen excretion, odors and methane emissions by up to 80%. Insects produce antimicrobial peptides to protect themselves from microbial infections, and these peptides may also be functional in poultry feed. Feed containing antimicrobial peptides can improve growth performance, nutrient digestibility, gut health and immune function in poultry. Insect meal contains higher amounts of essential amino acids compared to conventional feeds.The exoskeleton of insects consists mainly of chitin. Chitin is known to enhance the immune system. Chitin and chitin derivatives are known to improve the immune system by stimulating the innate immune cells of poultry.

Compared with the alternative protein, the health, immunomodulatory and functional effects of insect flours come to the fore. Due to their high nutritional value and ubiquity, insects are a potential source of sustainable feed in poultry nutrition. However, the high cost of insect products and the risk of pathogenic contamination and disease are also significant disadvantages.

P42 Technical Investigation of Broiler Chicken Production in Manisa Province

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Abstract

This study aims to reveal the technical, structural and breeding characteristics of broiler farms in Manisa, the province with the highest production rate of 12.60% in broiler production, to reveal the problems faced by the producers and to identify the technical issues they need and to solve them. made to offer suggestions. The material of this research, 646 broiler farms within the borders of Manisa province, are divided into three groups as large (200 000 and over), medium (200000-100000) and small 100 000 and below, and 5, 10,10 'brokers from each group, respectively. The data obtained by the face-to-face survey method with the owners or responsible persons of each of the 25 broiler enterprises. When the enterprises participating in the survey are analyzed in terms of progress payment from integrated companies, 10 enterprises (40%) apply progress according to Feed Conversion Ratio (FCR), 12 enterprises (48%) European Production Efficiency Factor (EPEF). It has been determined that the other 3 enterprises (12%) progress payment according to (FCR+EPEF). When evaluated in terms of the litter material used in the broiler poultry houses, it was determined that 48% (13 farm) of the farms visited for the survey used rice husk, 32% (10) farm sawdust and 8% rice husk + zeolite. It was determined that 48% (12 farms) of the surveyed farms produced Ross 308 genotype, 44% (11 businesses) Cobb genotype, 8% (2 businesses) Hubbard genotype animals.

P43 Usability of Chitosan as a Feed Additive in Broilers

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Abstract

Chitin is the most common polysaccharide in the world after cellulose. An estimated 1 billion tons of chitin are to be produced each year in the biosphere. It is the primary component of cell walls in fungi, exoskeletons of arthropods such as crustaceans and insects, the radula of molluscs, cephalopods beak and back bones. It is also synthesized in small amounts by some fish and amphibians. It is complex and robust when chitin is mineralized with minerals, especially calcium carbonate. Chitosan and chitosan oligosaccharides are obtained by partially deacetylating chitin with strong alkalis such as sodium hydroxide. While chitin is insoluble in water, chitosan dissolves slightly in water and easily in acidic solutions, especially 2% acetic acid. The antimicrobial activity of chitosan has been proven against fungi, yeast and gram positive and gram negative bacteria. It is reported that 2 g/kg chitosan added to the feed significantly improved the feed conversion rate. This chitosan level also increased the ratio of villus height and crypt depth, while tended to decrease ammonia nitrogen in the colonic digestive tract. Chitosan as a feed additive is increasingly attractive due to its complete biodegradability, no residue in the products of the animals added to the feed or water, non-toxicity, antimicrobial properties and other effects.

Keywords: Chitosan, feed, water, feed additive.

P[44] Effects of Competitive Exclusion on Gut Microbiota of Broilers

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Absract

The intestines have a substantial effect onthe healthof broiler chickens in addition to being the place where nutrients are digested. Anydisordersinthe intestinemayaffect both the health and performance in broiler chickens. A healthy microbiota is thus substantial of importance for ensuring efficient broiler production. With the prohibition of the use of antibioticsas growth factor in broiler nutrition,competitive exclusion practices are being used in order to improve gut health. Competitive exclusion applications may reduce pathogenic bacteria throughmechanisms such as the production of bacteriocins and organic acids, competition for the nutrients available in the gut, competition for the attachments on the gut enterocytes,and subsequently improve gut microbiota.

Keywords: broiler, competitive exclusion, intestinal microbiota

P45 Effect of Feeding on Ascites Formation in Broilers

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Abstract

Ascites is a non-infectious metabolic disorder characterized by excessive fluid accumulation in the abdominal cavities of broilers and remains a global problem. The incidence of ascites in broilers is generally related to the growth rate. Some feeding methods reduce the incidence of ascites. These methods focused on to limit the level of feeding and improve general health. However, it is known that essential nutritional factors such as rations with high nutrient density, high feed intake and feed form affect ascites formation in broiler chickens. This review has given information about the factors affecting the appearance of ascites and the measures to prevent ascites.

Keywords: Broiler, ascites, nutrition, ration

P[46] Comparison of Animal Welfare Levels of Slow and Fast Growing Broiler Genotypes Raised Under Commercial Conditions

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Abstract

The welfare of broilers is both an important economic issue and an important criterion in terms of the five freedoms that animals should have. This study was conducted to compare the animal welfare levels of slow (Hubbard JA57) and fast growing (Ross 308) broiler chickens reared under commercial conditions. In the experiment, a total of 200 slow and fast growing broilers at the age of six weeks, 50 females and 50 males (2x2) from each group, were used. At the end of the period, the animals were scored for footpad lesions, hock joint lesions, feather cover level, feather dirtiness level and gait scoring. The findings were compared with the General Linear Model analysis method using the SPSS program. In general, it was concluded that the animal welfare levels of fast growing broilers were worse than slow growers. While the hock joint lesion was more severe in male animals, the level of breast dirtiness was more severe in female animals. Genotype x Gender interaction was generally found to be insignificant. As a result, it was concluded that monitoring the animal welfare levels of rapidly growing broiler chickens and the factors affecting it more carefully is important both economically and in terms of reducing the pain level of the animals.

P47 Efficacy of Different Zinc Sources in Broiler Production

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Abstract

The essential trace element zinc (Zn) is a common feed additive in animal nutrition. Zn is added either in inorganic or organic form. Literature reports differences between the two sources in availability, as well as in effects on animal performance. Although the results are not always consistent, organic trace elements are generally considered superior (Schlegel et al., 2013). The nature of the chemical bond in the organic trace elements differentiate them from one another. While monoglycinates are complexes in which one amino acid (AA) is bound to a metal ion, bis-glycinates are chelates in which two AAs are bound to a metal ion. The objective of this study was to determine the efficacy of different Zn sources in broilers. For the study, 512 day-old male broilers (Ross 308) were randomly assigned to one of four treatments (8 replicates; 16 birds/repeat; caged): T1) no added Zn, T2) 80 mg/kg Zn from ZnSO4, T3) 40 mg/kg Zn from Zn monoglycinate, T4) 40 mg/kg Zn from Zn bisglycinate, The diet consisted of corn, barley, and soybean meal. On day 35, performance parameters (BW, BWG, FCR) were numerically, but not statistically, best at T4, followed by T3 > T2 > T1.

The results of this study show that organic zinc sources, delivering an amount of Zn equal to 50% of the inorganic zinc sulphate- dosage, perform equally or numerically better than the latter.

Introduction

Zinc (Zn) is involved in numerous physiological processes from growth to protein synthesis, and bone metabolism. Muscles and bones are the tissues accounting for the largest proportion of Zn in the body (Suttle, 2010). Because the natural Zn content in the diet is often inadequate to meet the animal's needs or the Zn contained in the diet is not sufficiently available to the animal, Zn is supplemented. Zn sources can be inorganic (e.g., Zn sulphate, $ZnSO_4$) or organic (e.g., amino acid chelates). The aim of this study was to evaluate the efficacy of inorganic (Zn sulphate) and organic Zn supplements (Plexomin® Mono Zn 26 or Plexomin® Bis Zn 29) on growth performance and Zn digestibility in broilers.

Material and Method

512 day-old Ross 308 male chickens were distributed (according their average weight) into 4 treatments. Experimental basal diets (based on corn, barley and soy bean meal) were formulated as per breeder's recommendation to provide all nutrients except mineral Zn. The treatments were: 1) No Zn supplementation (negative control), 2) 80 ppm Zn from inorganic source (sulphate), 3) 40 ppm Zn from organic source (Plexomin®Zn 26) [Monoglycinate], 4) 40 ppm Zn from organic source (Plexomin® Bis Zn 29) [Bisglycinate]. Each treatment consisted of 128 chickens kept in cages of 16 individuals each.

Body weight, weight gain, feed consumption, feed conversion ratio (FCR), feed efficiency (FE) and mortality were measured for each cage.

The temperature was maintained at 35°C for the first seven days and then gradually decreased to 27°C (over two weeks) and maintained until the end of the study. The broilers in this experiment received 23 hours of light and one hour of darkness per day.

Findings

BW, BWG, FI, FCR and mortality did not differ significantly between treatment groups.

Results and Discussion

The table below shows the results of the measured performance parameters.

Measurements	T1-No Zn	T2-80 ppm $ZnSO_4$	T3-40 ppm Plexo-min® Mono Zn 26	T4-40 ppm Plexo-min® Bis Zn 29	LSD	P
0-35 Days						
Live body weight (g)	2262	2280	2294	2297	97.21	0.79
Weight gain(g)	2226	2244	2258	2261	97.29	0.79
Feed intake per bird (g)	3311	3303	3267	3248	0.122	0.76
FCR (g feed/g gain)	1.488	1.474	1.448	1.438	0.056	0.30
Mortality (%)	0	0	0	0	0	-
ZnDigestibility (%)	20.02	22.68	22.35	23.00	3.57	0.28

The results of this study show that the organic Zn sources (T3 & T4) did not differ significantly, but numerically outperform the other treatment groups (T1 & T2). It should be mentioned here that in the organic treatment groups, the Zn concentration was half that of the T2 treatment group, i.e., same level of performance (or numerically better) with half the Zn dose of Zn sulphate in mono- and bisglycinates. The results of a study by Feng et al. (2009), with broilers and different Zn sources also showed significantly improved performance of the Zn glycinate groups over the Zn sulphate group. These results are supported by a literature review by Stuchlich (2019), who concluded that in most studies, organic Zn sources perform better in poultry than inorganic Zn sources.

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P48 Efficacy of Methionine Hydroxy Analogue-Free Acid in Comparison to DL-Methionine in Broiler Chickens

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Abstract

This study was conducted to investigate the efficacy of methionine hydroxy analogue-free acid (MHA) relative to DL-methionine (DL-Met) in broilers by measuring growth performance, carcass traits, relative bioavailability (RBV) of Met and gene expression in liver. A total of 792 one-day-old male chicken were allocated into 7 dietary treatments consisting of corn-soybean meal based-basal diet without any Met addition and basal diet supplemented with low, recommended and high level of additional methionine corresponded to 25, 100 and 125 % of required Met addition, respectively to meet digestible Met+Cys requirements either from DL-Met or MHA (added about 1.5 times higher than the amount of DL-Met due to assumed RBV of 65% on product basis).

Regardless of source, methionine supplementation enhanced ($P<0.05$) growth performance, carcass and breast meat yield as no further improvement with the highest level of addition. Relative weights (% of body weight) of liver and pancreas were significantly decreased with the supplementation of both methionine sources. No statistically significant differences between methionine sources were observed in growth performance, carcass traits and gene expression in liver ($P > 0.05$) except expression of growth hormone receptor mRNA on which a significant interaction between source and addition level was observed. Compared to DL-Met, RBV of MHA was found 79.8 %, 62.8 % and 55.3 % for body weight, FCR and breast meat yield, respectively without any significant difference between methionine sources ($P > 0.05$).

Overall, it is concluded that MHA can be as efficacious as DL-Met in broiler chickens when approximately 1.5 times higher amount of MHA is added to corn-soybean meal based diets.

Keywords : broiler, methionine, relative bioavailability

Introduction

Methionine (Met) is an essential amino acid that is required for protein synthesis, and is the first limiting amino acid in corn-soybean based broiler diets. Supplemental Met sources are thus provided in the broiler diets. Crystalline DL-methionine (DL-Met; 99% of active substance) and DL-methionine hydroxy analog free acid (MHA; 88% of active substance) are the main feed-grade Met sources, and widely used in broiler feed production. MHA has a hydroxyl group on the alpha carbon instead of an amino group and is thus not an amino acid but rather Met precursor. MHA needs to be converted to biologically active L-Met for utilization in the metabolism. Thus, the efficacy of MHA as a source of methionine in broiler nutrition is a subject of ongoing debate. Even though a large number of studies have been conducted to compare the efficacy of MHA relative to DL-Met in broilers during the last decades, there is still a great deal of controversy in this subject. Some researchers revealed that both Met sources have the similar efficiency on an equimolar basis for broiler performance and carcass traits [1, 2] while the others showed that MHA was markedly inferior to DL-Met [3]. Likewise, the results of relative bioavailability (RBV) are inconsistent [4-6] in the literature, and a lack of research on gene expression also hinder thorough evaluation of the efficiency of MHA over DL-Met.

Therefore, this study was conducted to investigate the efficacy of DL-methionine hydroxy analogue-free acid (MHA) relative to DL-methionine (DL-Met) in broilers by measuring growth performance, carcass traits, relative bioavailability (RBV) of Met and gene expression in liver.

Materials and Method

A total of 792 one-day-old Ross 308 male broiler chicks were weighed and then randomly allotted to 7 treatments with 7 replicates (except basal diet which has 6 replicates, in total 48 replicates/floor pens) using a $2 \times 3 + 1$ factorial arrangement in a randomized complete block design with a common basal diet.

Dietary treatments were composed of corn-soybean meal based-basal diet without any Met addition and basal diet supplemented with low, recommended and high level of additional Met corresponded to 25, 100 and 125 % of required Met addition, respectively to meet digestible Met+Cys requirements [7] either from DL-Met or MHA (added about 1.5 times higher than the amount of DL-Met due to assumed RBV of 65% on product basis).

Mash feed and water were supplied *ad libitum* and all birds were raised according to management practices in the guidelines [8] in an environmentally controlled poultry house.

Chicks and feed were weighed and feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were calculated per pen. Daily mortality was also recorded for each replicate. At the end of the experiment (day 40), 2 chickens per pen close to the average pen weight were selected for processing. Carcass, breast meat, drumstick, thigh, abdominal fat, pancreas and liver were weighed and relative weights (% of BW) were calculated. Dry matter, ash and crude protein analysis were carried out in *pectoralis major* [9]. Abundances of insulin-like growth factor-I (IGF-I) and growth hormone receptor (GHR) mRNA in the liver were determined using real time PCR and the relative quantification method [10, 11].

Pens were treated as experimental units and data for all response variables related to different phases of the trial were analyzed as a completely randomized block design, with a factorial arrangement of 2 × 3 + 1 for Met sources and its graded level, respectively, using the general ANOVA procedure implemented in SAS release 9.2 (SAS Institute, 2008). When significant differences ($P < 0.05$) were found between the groups, means were separated using the Tukey HSD test. Mortality data were subjected to the chi-square test. BW, FCR, and breast meat yield data were analyzed by non-linear multi-exponential regression for calculating the RBV [12].

Results and Discussion

During the whole growth period (0-40 days), supplementation from either source of Met increased feed intake, but extent of increase between the recommended and low level of supplementation was greater in DL-Met compared to MHA. There was no significant interaction between source of Met and level of supplementation in BWG and FCR ($P > 0.05$). Gradual significant improvements in BWG and FCR were obtained with increasing Met supplementation ($P < 0.05$) without any further enhancement with the high level of addition. Met source however had no significant effects on these parameters. Mortality was not significantly affected by any of the factors studied. These results are in line with some researchers indicated that regardless of source Met supplementation to Met deficient diets improved growth performance in broiler chickens [13]. Higher carcass and breast meat yield compared to basal diet were also obtained with Met addition as no significant difference between DL-Met and MHA in the same study [13]. Likewise, supplementation of either Met source significantly increased carcass and cuts yield in the current study. No further improvements were observed in carcass, breast meat yield and relative weight of thigh above the recommended and low levels of Met supplementation, respectively. Relative weights of abdominal fat, liver and pancreas were not significantly affected from Met source ($P > 0.05$). Broilers that were fed diets with low levels of Met supplementation had higher relative weights of abdominal fat compared to those with recommended and high levels of Met addition. Similar result was observed by [2], and this may be related to lipotropic effects of Met due to its role in the metabolism. In the current study, with Met supplementation, the relative weights of the liver and pancreas were gradually reduced compared to the basal diet, except in cases of high-level addition, which showed no difference compared to the recommended level of addition. This may be attributed to elevated amino acid metabolism mainly induced by an imbalance in the Met:Lys ratio in Met-deficient diets as indicated by [14]. Dry matter, crude protein, crude fat and ash were not significantly affected by any of the factors studied ($P > 0.05$). The RBV of MHA compared to DL-Met estimated in the present study for BW, FCR and breast meat yield of broilers was 79.8 %, 62.8 % and 55.3 %, respectively, without any significant difference between the Met sources. In the current study it was showed that the addition of DL-Met to the basal diet, at recommended and high levels, significantly increased the expression of GHR mRNA in the liver of broilers. However, only the recommended level of MHA addition resulted in a significant increase. Expression of IGF1 gene was not significantly affected by either Met source or supplementation level ($P > 0.05$).

Conclusion

Overall, when about 1.5 times higher amount of MHA is added to corn-soybean meal based diets, no significant differences were observed between MHA and DL-Met as Met sources for growth performance and carcass traits in this study. Furthermore, RBV of MHA for BW, FCR and breast meat was not significantly different from that of DL-Met. Based on these findings it can be concluded that DL-Met could be substituted with about 1.5 times higher amount of MHA addition in corn-soybean based diets without any compromise in growth performance and carcass traits in broiler chickens.

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P[49] Synergetic Effects of Medium Chain Fatty Acids and Phytobiotics in Broilers

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Abstract

In this study, we aimed to evaluate the effects of medium chain fatty acids (MCFAs) or phytobiotics blended feed additives on the broiler performance, cecal microflora and gut morphology. For this purpose 765 day-old male Ross-308 broiler chicks were randomly divided to five experimental treatments consisting nine replicates. The experimental treatments were allocated as a control diet or control diet (T1) supplemented with a blend of MCFAs and/or phtobiotics (essential oils (EOs) and alkaloids (ALKs)). Treatments were allocated as a T2 with a blend of MCFAs and EOs, T3 with a mixture of different EOs, T4 with a mixture of different EOs and ALK sanguinarine and T5 with a mixture of different EOs and ALK piperine. As a result of the study, broilers fed a diet supplemented with MCFAs blended with EOs had significantly improved body weight gain (BWG), jejunum morphology, caecum *Lactobacillus spp.* count and propionate, acetate and butyrate concentration of caecum comparison with control treatment. Hence, it can be concluded that the MCFAs and EOs combination may use for improve the growth performance of broilers by positively influencing gut morphology, SCFA production and caecum microbiota composition via strong synergism between them.

Introduction

Poultry gut health has become one of the most important issues in conventional poultry production. Escherichia coli, Salmonella, *Campylobacter*, Clostridium and Eimeria spp. are leading pathogens that pose significant threats to poultry gut health, resulting in performance and economic losses. Once these bacteria gain entry into the animal via feed, water and air, mainly colonising the caecum, pathogens spread through the alimentary canal, interfere with the gut environment and microflora and, subsequently, prevent chicks from reaching their genetic growth potential (1, 2, 3). Antibiotic growth promoters (AGP) have been utilized in broiler nutrition to alleviate the negative effects of the pathogenic microbes to promote the growth of birds due to enhancing effect on GIT microbiota, reducing bacterial fermentation, decrease the thickness of the GIT wall and suppress microbial dissimilation and control outbreaks of enteric diseases (2). However, following the EU and US bans on the use of AGP, because of the increasing emergence of antibiotic resistance in pathogens identified as public health risks (3) several approaches, such as organic acids, herbal extracts and oligosaccharides have been investigated for their benefits to improve poultry performance by reducing pathogens in chick intestines and improving gut integrity at the same time. Thus, this is an ongoing issue to find out better solutions for broilers to overcome the above-mentioned challenges related to microbiota and intestinal integrity (4, 5). One of them -medium chain fatty acids- (MCFAs) are among the most promising as an option to antimicrobial utilization in animal due to their nutritional, physiological and antimicrobial properties (6) and have strong antibacterial effects due to their capability to cross bacterial membranes (7). Besides phytobiotics or botanicals as another most promising alternatives are composed of natural bioactive components of plant origin, including terpenoids, alkaloids, glycosides and phenolic (8). Therefore, this study aimed to investigate the comparative effect of commercially available products of MCFAs, EOs and ALKs alone or along with each other as natural supplements recommended at the practical conditions on growth performance, cecum microbiota, volatile fatty acid production and jejunum morphology of chickens.

Material and Methods

Birds and Housing :The research was carried out in the Poultry House of Animal Science Department, Ankara University. 765 day-old male Ross 308 chicks were used as animal material and trial was conducted according to completely randomised block design. One-day-old chicks were randomly distributed into five dietary treatments each has nine replicates with 17 chicks in floor pens of poultry research unit. Rearing conditions were set according to breeder guidelines (9). Chicks were allowed ad libitum access to feed and water. Three phase feeding program was administered through the experiment as starter (0-10 days), grower (11-24 days) and finisher (25-41 days).

Diets : Diets for each feeding period were formulated to meet or exceed breeder guidelines and treatments consisted different feed additives supplement while feeding process. All of the diets were formulated based on corn, soybean and wheat and starter feeds of all the treatments were produced as crumble form while grower and finisher feeds were pellet form. Treatments were consisted as follow: T1: Control (without MCFAs, EOs or ALKs supplementation), T2: Control diet supplemented with MCFAs and essential oils based additive (M-prove®Poultry), T3: Control diet supplemented with mixure of different essential oil based additive (BIOSTRONG®510), T4: Control diet supplemented with ALK sanguinarine (Nusan 1500) and T5: Control diet supplemented with EOs and ALK piperine based additive (CRINA®Poultry Plus).

Measurements : All chicks were weighed at first, 11th, 25th and 41st days of the research and live weight and feed gain determined. Feed Intake was recorded for 0-11, 11-25 and 12-41 days. Mortality was recorded on a daily basis. FCR was calculated for 0-11, 11-25 and 12-41 days. At the end of the trial 2 broilers from each pen was selected to assess carcass, drumsticks, breast meat yield. Carcass, drumsticks (with bones), and breast meat (with bones) were excised and weighed, then calculated as a percent of live body weight. In order to determine the morphological parameters of the each slaughtered birds, 5 cm-long-parts of the jejunum were taken and images were taken with a computer-supported imaging system and jejunum morphology determined. Short chain fatty acid (SCFA) concentration was determined by gas chromatography (10) and cecal microbiota was analysed using culture technique (11).

Statistical Analysis : All data originated in the current study were determined by ANOVA using the General Linear models procedure of MINITAB®18 software (Minitab Ltd., Coventry, UK) in a randomized complete block design, and mortality data were subjected to a chi-square test. Probability values less than and equal to 0.05 were considered significant and differences among treatments were separated by Duncan's multiple range test.

Results

Broilers fed a diet supplemented with MCFAs blended with EOs group (T2) had significantly greater body weight gain during overall period compared to control and T3 groups, while better FCR compared to the control. Moreover, while the other phytobiotic-based additives had no influence on the jejunum morphology compared with the control MCFAs blended with EOs to broiler diet significantly increased villus surface area, villus height and villus height/crypt depth ratio of jejunum in comparison with the control group. Besides, the MCFAs blended with EOs product in broiler feed significantly increased the acetate, propionate and butyrate concentration and *Lactobacillus spp*. population in the cecum and decreased the concentration of branch chain fatty acids (BCFA). ALK sanguinarine supplementation also significantly elevated the acetate, propionate and reduced the BCFA concentration in the cecum.

Figure 1: Effects of different herbal feed additives supplementation on final body weight gain (BWG) and feed conversion ratio (FCR)

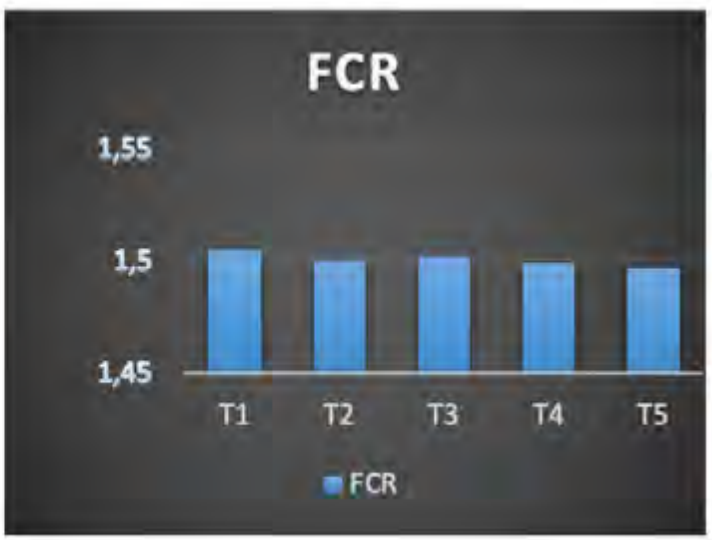
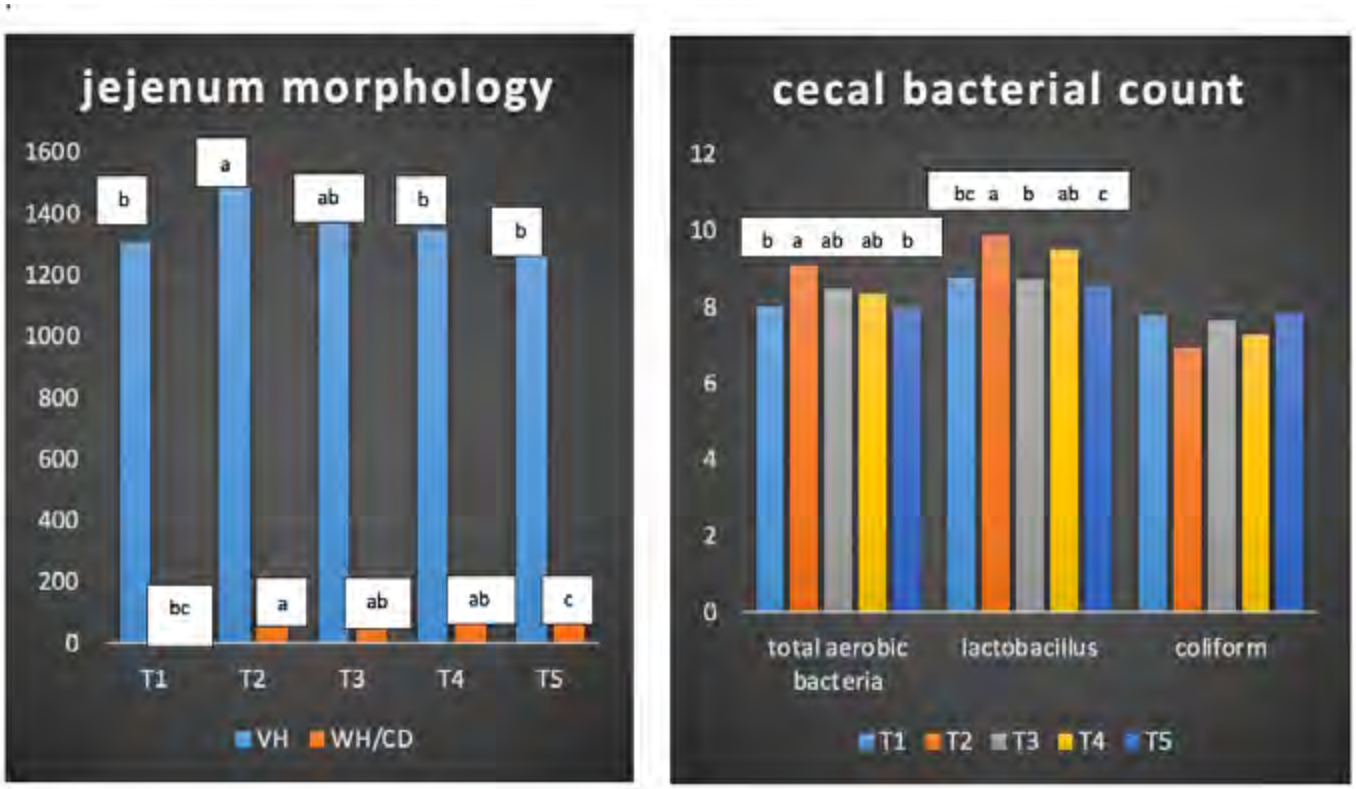


Figure 2: Effects of different herbal feed additives supplementation on some jejnum morphplogy (µm) and cecal bacterial count [log (cfu/g)].



Discussion

In our study, we found that, synergistic effects of MCFAs combined with EOs on broiler production in terms of improved final BWG and FCR. In line with our study, similar synergetic effects and increases in performance parameters also mentioned in other studies conducted by many researchers (12, 13, 14). Besides, in our study we also found that villus height, villus surface area, VH/CD ratio and number of goblet cells in the jejunum were highest with the application the MCFAs. In line with our results, previous studies (15, 16, 17) also are in agreement with the our findings and indicated that these improvements might increase the nutrient absorption and disease resistivity. As a results of the improvements in caecum morphology, body weight gain was might affected positively. In addition, increased morphological structure of the jejenum with the addition of MCFAs and EOS combination may be attributed to the synergetic action of MCFAs and EOs related to their strong antimicrobial activities on pathogens, and the nutritional contribution of MCFAs to epithelial cells of the intestine. In addition, the increased concentration of acetate, propionate, and butyrate, and reduced BCA concentration in the caecum may be further associated with the antimicrobial properties of MCFAs-EOs blend inclusion in this study by limiting the growth of pathogens, but promoting the beneficial bacterial colonization in the gut. Moreover, it can be speculated that the synergistic effects between the MCFAs and the EOs by modifying the gut microflora in favour of beneficial bacteria such as *Lactobacillus spp*. could result in the increased concentrations of acetate, propionate butyrate. This result is in accordance with that reported by Meimandipour et al. (18) who suggested that lactate, produced by Lactobacillus spp. in the cecal digesta, promotes the growth of butyrate-producing bacteria, which notably increases the cecal butyrate concentration.

Conclusion

The results of this study clearly showed that the medium chain fatty acid combined with essential oils significantly improved the growth performance of the broiler chickens starting from the starter period in comparison to the unsupplemented control and other phytogenic additives, which was supported by not only by improved balance of gut microbiota in favour of *Lactobacillus spp*., but also the improved surface area of jejunum, and SCFAs, mainly butyrate produced by caecum bacteria.

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P[50] Effect of Ca Levels on Performance, Bone Ash and Ileal Digestibility of Ca and P in Broilers Fed Phytase and Endo-β1,4-Xylanase

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Abstract

Reducing the Ca levels had a large positive impact, not only on animal performance, but also on Ca and P digestibility, and thereby on the efficiency of OptiPhos® Plus, yielding higher P matrix values. Reducing Ca levels might impact bone formation slightly, showing that Ca requirements for bone formation might be higher than Ca requirement for optimal animal performance.

Introduction

High levels of limestone have shown to have a negative impact on P digestibility, linked to the binding of Ca on the phytate molecule which limits the physical accessibility of the molecule by phytase. Particularly fine limestone, which shows high solubility seems to have a negative impact [1-5]. Therefor research is needed to find out what it the correct level of (digestible) Ca in the feed, in order to optimise performance without negatively affecting bone formation and broiler motility.

Materials and Methods

A broiler trial was set up (33 floor pens,12 male ROSS 308 broilers per pen) to investigate the effect of lowering Ca on performance, Ca and P digestibility and bone ash when fed a thermostable 6-phytase (OptiPhos® Plus). Birds were fed a starter (d1-8), grower (d9-21) and finisher feed (d22-35), to which 1000 FTU/kg phytase, and 1500 EPU/kg of an endo-1,4β-xylanase (Hostazym® X) was added (Table1). Levels of available P (aP) in starter, grower and finisher were 0.43%, 0.37 % and 0.22 %, using a contribution of 0.176 % aP from the phytase. Feeds were pelleted at ± 80°C (starter was crumbled); to the finisher TiO_2 was added at 0.4 %. Three treatments were implemented: normal Ca level (0.85, 0.7 and 0.6 %), reduced Ca level (0.7, 0.6 and 0.5 %), and low Ca (6.5, 5.0 and 4.0 %) in starter, grower and finisher respectively by reducing the inclusion of limestone (average particle size 0.39 mm; Table 1). Technical performance was measured after every feeding phase. At day 21, 3 birds per pens were killed and the right tibia was removed, pooled to one sample, and analysed for ash on fat free dry matter. At day 35, 9 birds of each pen were killed to collect ileal material for the determination of Ca, P and Ti, to calculate Ca and P digestibility.

Results

Reducing the Ca levels led to an increased technical performance, with the lowest Ca level giving a significantly higher end weight vs the normal Ca level ($p < 0.05$, Fig. 1). FCR was not impacted despite the higher end weight at reduced and low Ca levels. The Ca digestibility was highest at the lowest Ca inclusion level ($p < 0.05$, Table 2). The P digestibility was also highest at the lowest Ca inclusion level. Tibia ash, and Ca and P level in tibia ash, decreased with decreasing levels of Ca; this demonstrates that Ca availability, but not P availability, limits the bone formation. The fact that low Ca levels optimised technical performance, but tended to reduce bone ash, demonstrates that the Ca requirement for optimal bone growth is higher than the Ca requirement for optimal performance.

Conclusion

It can be concluded from this trial that at present Ca (limestone) is still overfed to broilers, and that lowering the Ca (limestone) level in broiler feed will enhance bird performance. However, although lowering Ca level in feed seems not to compromise performance, it reduces bone ash levels, to an extent it might compromise the birds motility.

Table 1. Feed composition and calculated nutrients

	Starter (d 1-8)			Grower (d 9-21)			Finisher (d 22-35)		
Feed materials (g/kg)	Normal Ca	Reduced Ca	Low Ca	Normal Ca	Reduced Ca	Low Ca	Normal Ca	Reduced Ca	Low Ca
Wheat	314	320	326	349	354	360	404	409	414
Corn	300	300	300	300	300	300	300	300	300
SBM 49	326	325	323	280	279	278	226	225	224
Soybean oil	17.5	15.8	14.0	34.5	32.8	31.0	37.3	35.7	34.0
Limestone	15.9	13.3	10.7	13.2	10.6	8.0	11.8	9.2	6.6
MCP	6.8	6.8	6.8	4.6	4.6	4.6	2.7	2.7	2.7
Others*	19.8	19.7	19.5	18.7	19.3	18.4	18.2	18.5	18.7
Formulated nutrients (g/kg)									
MEn broiler	2868	2867	2866	3022	3021	3019	3093	3092	3091
C. protein	224	224	224	205	205	205	185	185	185
Ca	8.5	7.5	6.5	7.0	6.0	5.0	6.0	5.0	4.0
P total	5.2	5.3	5.3	4.5	4.6	4.6	3.9	3.9	3.9
aP	4.3	4.3	4.3	3.69	3.7	3.7	3.2	3.2	3.2

* including salt, $NaHCO_3$, synthetic amino acids, mineral and vitamine premix, $Ti0_2$ as marker

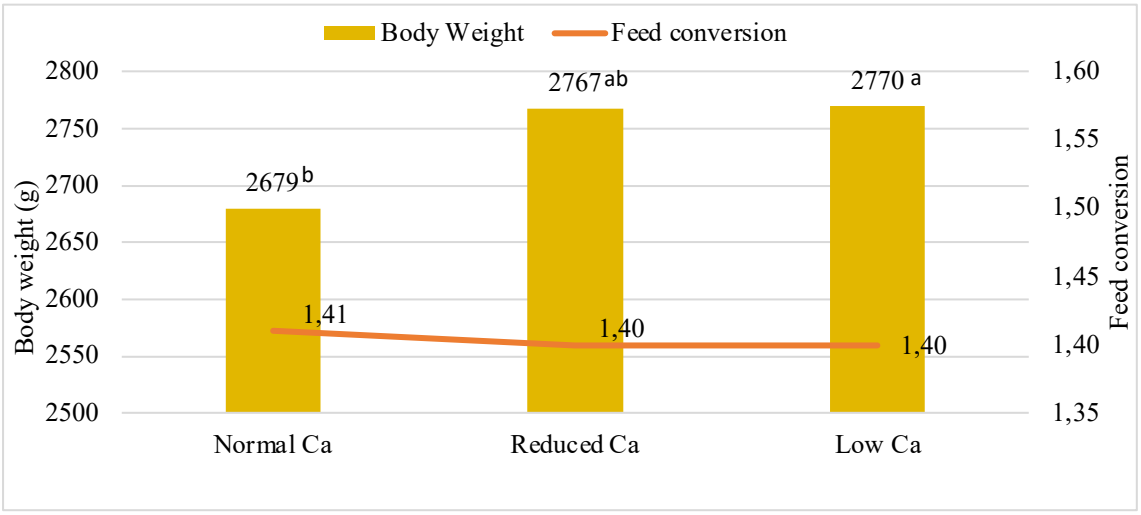

Figure 1. Effect of lowering Ca levels on body weight and feed conversion at day 35 (a, b values with a different superscript are significantly different at P<0.05)

Table 2. Effect of lowering Ca levels on digestibility of Ca and P, and on tibia ash content

Treatment	Ileal digestibility (%)		Tibia ash (% of DM)	Ca in tibia DM (%)	P in tibia DM (%)
	Ca	P			
Normal Ca	50.2^{b}	54.5^{ab}	48.4^{a}	18.6^{a}	10.1^{a}
Reduced Ca	53.9^{b}	51.3^{b}	47.6^{ab}	18.2^{ab}	9.9^{ab}
Low Ca	64.1^{a}	60.6^{a}	46.8^{b}	17.8^{b}	9.8^{b}

a,b: values in a column with different superscript are significant P different P < 0.05

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P51 Effect of a Novel *E. Coli* 6 Phytase on Broiler Performance, Bone Ash and Ileal Digestibility of Phytate, Phosphorus and Protein at Levels up to 1000 FTU/kg

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Abstract

In a broiler study, increasing levels of OptiPhos® Plus led to increased degradation of phytate-P, leading to an increase in available P by 1.00, 1.12, 1.54 and 1.53 g/kg feed at 250, 500, 750 and 1000 FTU/kg, respectively. Protein digestibility is enhanced up to 5 % by increasing levels of OptiPhos® Plus. This equals 1 % protein per kg feed savings assuming a feed protein level of 20 %.

Introduction

Plant seeds contain about 60-80 % of phosphorus (P) in is in the form of phytic acid. In the presence of with various cation sources such as $CaCO_3$ (as in feed) results in poor phytic acid degradation in poultry. A practical solution to this issue has been to feed microbial phytase as part of the feed mix to release P. However, the effects of phytase on nutrients such as protein are not consistently reported in the literature (the so-called 'extra-phosphoric' effects of phytase) [1-2]. Therefor it it is worthwhile doing P digestibility studies also to measure the effect on protein digestibility.

Materials and Methods

A broiler trial was set up (98 floor pens,17 male ROSS 308 broilers per pen) to investigate the effect of a novel thermostable 6-phytase (OptiPhos® Plus, Huvepharma NV) on performance, bone mineralization and ileal digestibility of total phosphorous (P) and crude protein (CP). All birds were fed a P sufficient starter diet (day 1 to 5) after which different grower feeds (day 5-21) and finisher feeds (day 22-35) were provided (Table 1). The starter feed contained 21.5% CP, 0.90% Ca and 0.45% available P (aP). The grower feed contained 20.5% CP, 0.80% Ca and 0.32% aP and the finisher feed contained 19.4% CP, 0.75% Ca and 0.30% aP. A negative control (NC) grower and finisher feed were produced by reducing 0.15% and 0.15% Ca and 0.17% and 0.15% aP from grower and finisher, respectively. To this NC, 0, 250, 500, 750 and 1000 FTU/kg of phytase was added. Feeds were pelleted at ± 80°C (starter was crumbled). Feed contained an indigestible marker (0.4% $Ti0_2$) to calculate digestibility. Technical performance was measured after every feeding phase. At day 21, 2 birds per pens were selected close to the pens' average weight and the right tibia was removed, pooled to one sample and analysed for ash on fat free dry matter. At day 35, 9 birds of each pen were killed to collect ileal material (from terminal ileum to 10 cm before the ileo-cloacal junction) for the determination of P, CP, and Ti. The body weight (BW) and feed conversion (FCR) for the NC were 1.58 kg and 1.57, respectively

Results

The body weight and feed conversion for the NC were 1.58 kg and 1.57, respectively. Adding phytase increased the BW and FCR in a dose-responsive way reaching 2.30 kg and 1.52 respectively at 35 days (1000 FTU/kg) while the PC reached 2.25 kg BW and a FCR of 1.54. Bone ash was increased from 36.9% to 45.9% at 0 and 1000 FTU/kg while the PC reached 47.9%. Protein digestibility in the PC and NC were 69.8 and 73.5%, respectively, while protein digestibility increased to 78.9, 79.2, 77.8 and 77.6% for 250, 500, 750 and 1000 FTU/kg OptiPhos® Plus, respectively.

Conclusion

Increasing levels of OptiPhos® Plus led to increased degradation of phytate-P, yielding a dig. P value of 1.53 g and an aP value of 2.04 g at 1000 FTU/kg inclusion level. Protein digestibility is enhanced up to 5 % by increasing levels of OptiPhos® Plus which equals 1 % protein per kg feed assuming feed protein level of 20 %.

Table 1. Feed composition and analysis

	Starter (d 1-5)	Grower (d 6-21)		Finisher (d 21-35)	
Ingredients		Negative control	Positive control	Negative control	Positive control
Corn	275	59	280	318	302
Wheat	300	300	300	300	300
Soybean meal 49 % CP	285	242	245	197	200
Rapeseed meal 33 % CP	30	40	40	60	60
Sunflower meal HP	20	30	30	30	30
Animal fat	10	40	40	40	40
Soybean oil	38.0	23.0	28.5	29.0	34.5
Limestone	14.5	13.6	14.0	12.0	12.9
MCP	14.2	1.0	8.6	1.2	7.8
Others*	5.0	5.0	5.0	5.0	5.0
Nutritional value (g/kg)					
Crude protein (g/kg)	216	204	204	191	191
Crude fibre (g/kg)	36	38	37	39	39
Crude fat (g/kg)	66	81	88	88	93
Crude ash (g/kg)	62	47	57	44	52
Starch (g/kg)	377	390	377	402	392
Dig. lysine (g/kg)	11.0	10.2	10.2	9.5	9.5
Calcium (g/kg)	9.0	6.5	8.0	6.0	7.5
Av. Phosphorus (g/kg)	4.5	1.5	3.2	1.5	3.0
Total P (g/kg)	7.2	4.2	5.9	4.2	5.7
ME (kcal/kg)	2985	3110	3109	3156	3156

* Salt, Sodium Bicarbonate, Synthetic Amino Acids and vitamin/mineral premix

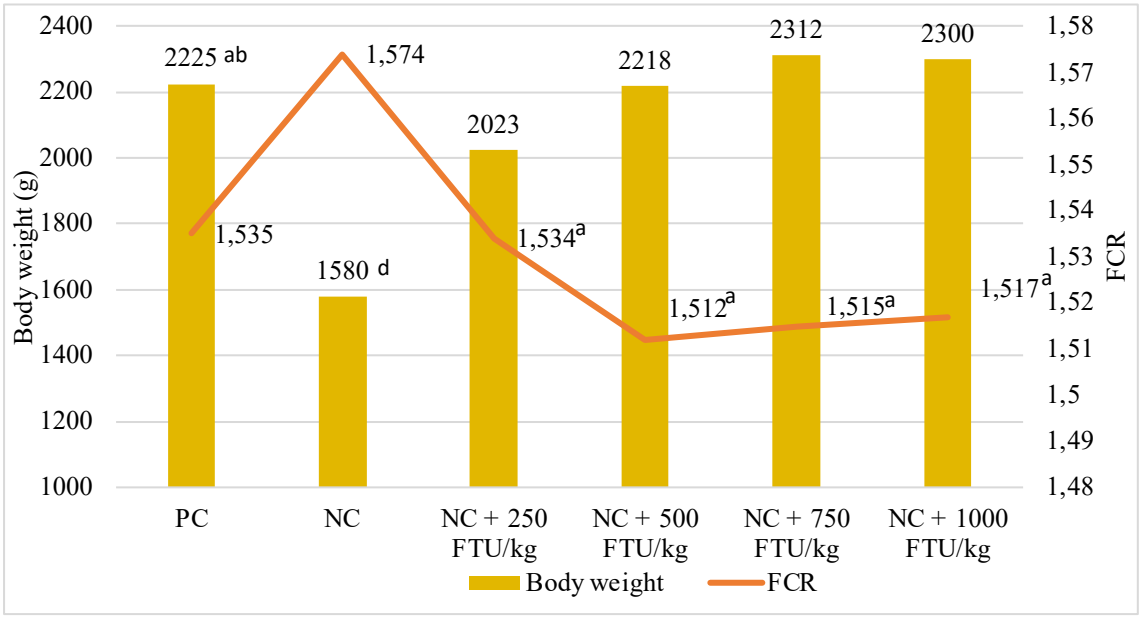

Fig. 1. Doses response of OptiPhos® Plus on performance (a,d values of line or column followed by different letter are sign. different ($p < 0.05$)

Table 2. Doses response of OptiPhos® Plus on bone ash and protein digestibility (%)

Treatments	Bone ash, %	Protein digestibility,%
PC	47.9a	73.5b
NC	36.9e	69.8c
NC+250 FTU/kg	40.1d	78.9a
NC+500 FTU/kg	42.8c	79.2a
NC+750 FTU/kg	43.8c	77.8a
NC+1000 FTU/kg	45.9b	77.6a

a,c: values in a column with different superscripts are sign. different ($p < 0.05$)

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P[52] Probiotic *Bacillus Licheniformis* DSM28710 Supports Broiler-Breeders under Commercial Conditions

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Abstract

The application of probiotics in broiler-breeders is underrepresented in available research, yet such an application should not be ignored. Probiotic *Bacillus licheniformis* DSM 28710 (B-Act®, Huvepharma) was put to the test under commercial conditions, showing clear benefits: animals that were initially underperforming were able to catch up to the control with the help of the probiotic, whilst they were better equipped to deal with stress situations. Simultaneously, a better functioning of the gastrointestinal tract resulted in a clear reduction of dirty eggs.

Introduction

Broiler applications dominate the probiotic research in literature. However, broiler-breeders should not be neglected: high-producing hens must also be as efficient as possible, including utilising their diet to the fullest whilst withstanding health challenges. From that perspective the importance of a proper functioning gastrointestinal tract cannot be underestimated, indicating the value a probiotic supplementation can bring to a broiler-breeder operation.

Materials and Method

To evaluate the effect of a probiotic *Bacillus licheniformis* strain in broiler-breeders, a commercial trial was set up. The strain used was DSM 28710 (B-Act®, Huvepharma) and applied via the feed from start to finish.

A total of 27 335 Cobb 500 broiler-breeders were included in the trial: 12 592 female and 973 male birds were assigned to the control group, with the probiotic group consisting out of 12 781 female and 989 male birds. All animals were 29 weeks of age at the start of the experiment, which lasted for 64 days (up until 38 weeks of age). The probiotic group was supplemented with 1.6 x 10^9 CFU *Bacillus licheniformis* DSM 28710 per kg of feed (500 g B-Act®/ton of feed, Huvepharma). Parameters recorded included laying percentage, hatchability and the amount of dirty eggs.

Results and Discussion

When the trial started, animals in the control group had a slightly higher laying percentage compared to the probiotic group (86.31 vs. 84.35%). This difference disappeared halfway the trial, with animals in the probiotic group catching up to the control due to the probiotic supplementation. In regards to hatchability, there was no difference during the trial period itself. That being said, a stress event occurred from week 40 until week 44. Hatchability reduced severely for 4 weeks, but animals previously supplemented with the probiotic *B. licheniformis* were more resilient (less of a reduction in technical performance) and were able to recover quicker compared to animals previously in the control group. In numbers: lowest hatchability for the probiotic group was 79% at week 41, with a partial recovery at week 42 (86.8%) and a full recovery at week 44. The control group had a low point hatchability of 74.4% at week 42, with a full recovery at week 44 as well.

In terms of dirty eggs for the trial period, animals supplemented with probiotic *B. licheniformis* DSM 28710 clearly benefitted from the addition to their diet: only 943 eggs were recorded as dirty eggs, compared to the control's 1780 – a reduction of almost 50%. This is in line with earlier research, conducted both in layers as well as broilers: both applications noted an improved functioning of the gastrointestinal tract, indicated by a reduction in dirty eggs (layers) and reduced wet litter (broilers). A secondary layer trial with the same probiotic also looked into manure parameters, including moisture and protein levels. A significant decrease in excreta moisture was noted in the probiotic group (moisture reduction of 5.3% compared to the control's 4.5%; $P<0.05$), which further lends credibility to the hypothesis that *B. licheniformis* DSM 28710 supports a better functioning of the gastrointestinal tract in layers and broiler-breeders. This includes an improved usage of the provided nutrients from the diet, as highlighted by the final manure parameter:

a significant reduction in manure protein in the probiotic group was noted (20.5 vs. 24.1% in the control, $P<0.05$), indicating that nutrients were utilised better when the probiotic was present.

Conclusion

The results here clearly show that *B. licheniformis* DSM 28710 supports high-performing broiler-breeders under stressful commercial conditions. As a result, animals were better equipped to deal with these conditions, whilst a better functioning of the gastrointestinal tract resulted in a clear reduction of dirty eggs. The results obtained under commercial conditions were in line with previous research on *B. licheniformis* DSM 28710 in layers and broilers, where similar improvements in secondary feed-efficiency parameters were noted. Probiotic *B. licheniformis* DSM 28710 should thus be included in diets formulated for high-performing broiler-breeders, to ensure optimal feed efficiency and animal resilience to stressful conditions.

P53 Effect of Two Different Dietary Plant Herbal on Growth Performance, Carcass Yield, Serum Biochemistry, Jejunal Histomorphometry, Oxidative Stability of Liver and Breast Muscle, and Immune Response of Broiler Chickens

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Abstract

Current study was carried out to determine the effects of two different dietary herbal plants on zootechnical performance, serum biochemistry, jejunal histology, oxidative stability of liver, breast meat, and immune response of Ross 308 broiler chickens. Three experimental groups were set as control, a commercial herbal alkaloid (60 g/ton), and Huverb (60 g/ton). All groups consisted of 5 replicates with 20 day-old chickens per replicate (totally 300 birds). Dietary treatments had no significant effect on growth performance. However, supplemental Huverb improved serum biochemistry. So, birds fed on diet containing Huverb significantly had the lowest ALT and AST on day 24 and 42 respectively ($P < 0.05$). There was no significant difference in the level of ALT between two feed additives on day 42 ($P > 0.05$). Huverb birds significantly had lower TBARS in liver on day 42, and freeze-thaw breast meat after 30 and 90 days of storage ($P < 0.05$). Antibody titer against infectious bursal disease virus was higher in birds received Huverb by 8.05 and 3.34 % compared to control and commercial alkaloid, respectively. It can be concluded that inclusion of Huverb in broiler diets can improve the health, oxidative stability of liver and breast meat, and immune response of broiler chickens.

Keywords: antioxidant, antibody titer, broiler, herbal plant, intestinal histology

Material Methods

was carried out to determine the effects of two different dietary herbal plants on zootechnical performance, serum biochemistry, jejunal histology, oxidative stability of liver, breast meat, and immune response of Ross 308 broiler chickens. Three experimental groups were set as control, a commercial herbal alkaloid (60 g/ton), and Huverb (60 g/ton). All groups consisted of 5 replicates with 20 day-old chickens per replicate (totally 300 birds).

Results

The results of the experiment were shown in Table 1, 2, 3, 4, and 5. Dietary treatments had no significant effect on growth performance. However, supplemental Huverb improved serum biochemistry. So, birds fed on diet containing Huverb significantly had the lowest ALT and AST on day 24 and 42 respectively ($P < 0.05$). There was no significant difference in the level of ALT between two feed additives on day 42 ($P > 0.05$). Huverb birds significantly had lower TBARS in liver on day 42, and freeze-thaw breast meat after 30 and 90 days of storage ($P < 0.05$). Antibody titer against infectious bursal disease virus was higher in birds received Huverb by 8.05 and 3.34 % compared to control and commercial alkaloid, respectively.

Table 1. Effect of dietary treatments on performance of broiler

Parameters	Control	Commercial Herbal Alkaloid	Huverb	P- value
BW, g				
d 10	272,3	289,8	296,7	1,33
d 24	1216,9	1231,3	1289,2	0,123
d 42	2665,6	2713,2	2798,9	0,304
BWG, g				
d 0-10	227,7	245,24	252,0	0,132
d 11-24	944,5	941	992,5	0,111
d 25-42	1448,0	1481,8	1508,7	0,604
d 0-42	2620,2	2668,2	2753,3	0,304
FI, g				
d 0-10	232,1	231,7	232,3	0,999
d 11-24	1119,4	1152,6	1175,9	0,06
d 25-42	2714,0	2795,8	2811	0,76
d 0-42	4066,0	4180,2	4219,0	0,605
FCR				
d 0-10	1,022	0,940	0,925	0,386
d 11-24	1,18	1,225	1,186	0,07
d 25-42	1,874	1,887	1,861	0,857
d 0-42	1,552	1,566	1,531	0,299

Table 2. Effect of dietary treatments on some blood parameters of broilers on d 42. (n= 20 birds/ treatment)

Parameters	Control	Commercial Herbal Alkaloid	Huverb	P- value
Total Protein d 24, g/dl	2,79	2,67	2,89	0,151
Albumin d 24, g/dl	1,36 b	1,48 ab	1,52 a	0,01
AST d 24, UI/I	300,21	313,34	265,45	0,105
GGT d 24, UI/I	15,95	16,20	13,75	0,103
ALT d 24, U/L	21,65 a	19,80 ab	16,15 b	0,001
Total Protein d 42, g/dl	2,95	3,12	3,22	0,184
Albumin d 42, g/dl	1,30 b	1,35 ab	1,46 a	0,012
AST d 42, UI/I	346,51 a	321,70 ab	273,82 b	0,008
GGT d 42, UI/I	18,75	17,20	16,65	0,389
ALT d 42, UI/I	22,60 a	19,25 b	18,20 b	0,001

[1] AST: Aspartate Aminotransferase; GGT: Gamma-glutamyltransferase; ALT: Alanine transaminase

[a-c] Means within the same row without common superscripts are significantly different ($P < 0.05$)

Table 3. Effect of dietary treatments on TBARS values of liver (mg malondialdehyde/kg) (n= 20 birds/ treatment)[1]

Parameters	Control	Commercial Herbal Alkaloid	Huverb	P-value
Liver TBARS d. 24	1,94 a	1,54 b	1,41 b	0,0001
Liver TBARS d. 42	1,84 a	1,68 ab	1,54 b	0,012

[a-b] Means within the same row without common superscripts are significantly different ($P < 0.05$)

[1] Stored frozen at -18ºC for 30 days; TBARS : Thiobarbituric acid reactive substances

Table 4. Effect of dietary treatments on TBARS values of breast meat (mg malondialdehyde/kg), carbonyl (nmol/mg protein) and sulphydryl (nmol/mg protein) in broilers (n= 20 birds/ treatment)

Parameters	Control	Commercial Herbal Alkaloid	Huverb	P - value
Fresh Breast Meat TBARS	1,32	1,27	1,23	0,26
Freeze Thawing breast meat TBARS; d 30 [1]	1,73 a	1,54 ab	1,42 b	0,006
Freeze Thawing breast meat TBARS; d 60 [2]	1,87 a	1,68 b	1,53 b	0,001
Freeze Thawing breast meat TBARS; d 90 [3]	1,87 a	1,69 b	1,55 b	0,001
Fresh Breast Meat Carbonyl	53,6	42,37	34,95	0,09
Freeze Thawing breast meat Carbonyl; d 90	82,38 a	57,92 ab	50,82 b	0,01
Fresh Breast Meat Sulphydryl	13,37	11,69	10,75	0,298
Freeze Thawing breast meat Sulphydryl; d 90	12,04	10,07	9,54	0,296

[a-b] Means within the same row without common superscripts are significantly different ($P < 0.05$)
[1,2,3] Stored frozen at -18ºC; TBARS: Thiobarbituric acid reactive substances.

Table 5. Effect of dietary treatments on intestinal morphometry of broilers on d 42. (n= 20 birds/ treatment)

Parameters	Control	Commercial Herbal Alkaloid	Huverb	P-value
Villus length, µm	1047,41 b	1219,24 a	1309,79 a	0,0001
Villus width, µm	145,58 b	168,64 ab	171,51 a	0,05
Crypt depth, µm	186,97	177,84	161,43	0,22
Villus length: Crypt depth	5,89 b	7,19 b	9,05 a	0,0001
Villus surface area, mm^2	0,57	0,69	0,59	0,11
Total goblet cell number	130,75 b	152,95 ab	160,15 a	0,013

[a-b] Means within the same row without common superscripts are significantly different ($P < 0.05$).

Conclusion

It can be concluded that inclusion of Huverb in broiler diets can improve the health, oxidative stability of liver and breast meat, and immune response of broiler chickens.

Acknowledgement

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P54 Determination of Vaccination Success of Broilers Vaccinated by Different Methods in Incubation Against Infectious Bronchitis and Newcastle Disease by Molecular and Serological Methods

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Abstract

Infectious bronchitis (IB) and Newcastle disease (ND) are very contagious viral diseases that affect the respiratory system in broilers and cause serious economic losses. In this study, it was aimed to determine the success of vaccination by molecular and serological methods in broilers vaccinated against infectious bronchitis and Newcastle disease with different methods in the hatchery. For this purpose, 61,820 broiler chicks vaccinated with eye drop method and 86,740 broyler chicks using spray vaccination method were used in the hatchery. On the 10th day, 168 blood serum and 60 trachea swab were taken from these herds. While blood sera were analyzed by hemagglutination inhibition (HI) test, swabs were analyzed by quantitative real-time PCR. When the findings were evaluated, the IBV titer obtained as a result of the HI test in the first group in which eye drop vaccination was applied, was found to be 5.19, and the IBV titer obtained as a result of the HI test in the second group was 4.29. Similarly, NDV titer was 5.54 in the first group and 4.48 in the second group. Molecular findings also supported these results. In the first group, 93.3% positivity was found in the analysis for IBV, while the positivity rate in the second group was 73.3%. While 90% positivity was found in the analysis for NDV in the first group, the positivity rate in the second group was 76.6%. As a result, it was determined that the eye drop vaccination method was more effective than the vaccination with the spray booth, both serologically and molecularly.

Keywords: Infectious bronchitis disease, Newcastle disease, Vaccination efficacy, vaccination method

P[55] Potential of Spirulina as Broiler Feed Additive

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Abstract

Microorganisms, which have the ability to synthesize nutrients that form the basis of nutrition, such as carbohydrates, proteins, lipids, minerals and vitamins, from organic and inorganic substances, gain great importance to solve the problem of food insufficiency in the world. Spirulina contains high crude protein (>60% dry matter content) essential amino acids, fatty acids, vitamins and minerals. It is also rich in omega-3 polyunsaturated fatty acids, vitamin B12, carotenoids and antioxidant compounds. The increasing demand for animal-derived proteins and the need for large amounts of protein supplement feed required for production encourage research on the use of spirulina as a sustainable source in animal nutrition. In this review, hundreds of studies including the words spirulina, broiler and growthperformance have been scanned and the results of studies involving *in vivo* experiments in the last 5 years have been summarized. It was reported that the addition of Spirulina positively affected the growth performance in broilers in 14 of the 15 studies examined, and the growth performance was not affected in 1 of the 15 studies under heat stres conditions.

P56 Sustainable Innovative Technologies in the Evaluation of Wastes in the Poultry Meat Sector

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Abstract

In our world, where access to raw materials is increasingly restricted, innovative technologies are used in line with environmental policies. The role of sustainability in this regard is undoubtedly huge. Hatchery waste, dead chickens, slaughterhouse and feed industry wastes and wastewater are the main wastes generated in the poultry industry. In the food sector, where the consumption demand is increasing, the tendency to consumption of poultry meat is increasing and the evaluation of the wastes generated is of great importance. In this direction, various technologies such as fertilizer for plants, feed for animals, bioenergy and biogas production for industry are being developed from waste mass.

Keywords: Sustainability, poultry waste, development plan, waste management

P57 Evaluation of Onion Juice in Chicken Doner Marination

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Abstract

Recently, due to the rapid population growth and the post-pandemic global crisis and the difficulty of obtaining red meat, consumer demands for chicken meat consumption to meet the need for animal protein have increased. Chicken meat has been preferred because of its richness in essential amino acids, high bioavailability and its cost advantage at the same time. Especially in recent years, the advantages of chicken meat, which has many alternative product types in the market as whole and piece, such as providing a time advantage in terms of easy preparation and being available as cooked or half-cooked as well as the processed product variety, have increased the tendency of the consumer to these products. One of the most demanded products among chicken meat products is doner. Although doner is a product belonging to the Middle East culture, it is lovingly consumed in all countries of the world. The meat prepared for döner is opened with a knife to suit the wrapping and then marinated in the marinating solution prepared with salt, spices, onions, yogurt and oil. The marination process can be done with immersion, which is a classical method, or it is done with injection or drumming techniques in industrial size. The primary purpose of this process is to give the meat flavor and tenderness. The different properties of the raw materials used together are also changed the product characteristics. Onion, which is the basis of main dishes in Turkish cuisine, are also frequently used in marination processes. The structure of the onion contains valuable bioactive compounds such as flavanoids and alkein cysteine sulfoxides. Alkenyl cysteine sulfoxides are the flavor components that are broken down by the enzyme allinase to form the unique smell and taste of the onion. It has been established that the compounds of allicin and thiosulfate, which are present in the structure of the onion, exert an antimicrobial effect. These allium-derived antimicrobial compounds inhibit microorganisms by reacting with sulfhydryl (SH) groups of cell proteins. Allicin and other allium-derived compounds have been found to exert inhibitory effects against all microorganisms, including bacteria, fungi, viruses and parasites. In this study, the use of waste onion water in the production of chicken doner was investigated in order to contribute to the sustainable production process, which is a popular topic today, as well as providing flavor development and microbial safety. The effect of onion juice (1.3%, 1.8% and 2.4%) added to the marinating solution in different proportions in the production process on the physicochemical, microbiological and sensory properties of chicken doner was examined.

Keywords: chicken doner, onion water, flavor development, microbial safety

P58 Are Probiotics a Real Alternative Feed Additive to Fight the Invisible Enemy *Campylobacter* in Broiler Houses?

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Abstract

Campylobacter spp. are zoonotic bacteria that are the main cause of foodborne gastrointestinal infections known as campylobacteriosis, affecting the health of large populations worldwide and threatening food safety. Most domestic animals are the natural host of *Campylobacter*. However, poultry meat is considered the most important vector of *Campylobacter* from farm to table. Broiler houses provide an optimal environment for *campylobacter* proliferation and after contamination, the pathogen spreads rapidly and colonises the gastrointestinal tract of the birds. However, chickens no indicate any clinical signs and detection is not possible without time-consuming laboratory techniques. Strict biosecurity precautions and the use of antibiotics can prevent *Campylobacter* infection in broiler houses, but antibiotic resistance in bacteria raises food safety concerns. Therefore, alternative nutritional approaches have recently emerged as a substitute for antibiotics to control *campylobacter* in broiler production. Recently, probiotics, which are beneficial bacteria for humans and animals, have been considered as a promising antibiotic alternative and feed additive to combat pathogenic bacteria, and numerous studies have been conducted on the effects of probiotics on broiler performance, pathogenic bacteria, the immune system, etc. However, the mechanism of action of probiotics against pathogens, including *Campylobacter*, is still unclear and experimental evidence is needed to evaluate probiotics as an effective means of controlling *Campylobacter* in broiler production. Therefore, this study scanned the experimental *in vivo* research on the use of probiotics against *Campylobacter* in broiler feed or water over the last 5 years and the results were summarised. In summary, despite a large body of research on the use of probiotics in broilers, few of them have investigated the effects of probiotics on *Campylobacter* counts in broilers *in- vivo*. In five out of a total of 11 experimental studies, a significant reduction in *Campylobacter* counts in broiler samples was found as a result of the administration of probiotics. In a conclusion, there is evidence that probiotics can reduce *Campylobacter* colonisation in broilers. However, experimental studies are not adequate to infer and declare probiotics to be effective against *Campylobacter* in broiler production.

Introduction

Campylobacter is a thermophilic, Gramme-negative, comma-shaped, motile bacterium and a zoonotic agent that can colonise the intestinal mucosa of most warm-blooded animals (1). A total of 32 species and 9 subspecies campylobacter genus have been reported (2), but, identified strains of campylobacter of broiler carcasses have been as about two-thirds is *Campylobacter* jejuni and one-thirds is *Campylobacter* coli (3, 4). C. jejuni is considered to be part of the normal intestinal flora of poultry due to its high adaptability to the host, and large numbers can be detected in the intestine and faeces after a single infection without any apparent clinical consequences for the chickens (5),On the other hand some researchers reported that *campylobacter* jejuni cause enteritis and detrimental effect on growth and increase feet and leg problem in broiler chickens (6). However, Campylobacter is pathogenic to humans and Campylobacteriosis is one of the most common gastrointestinal diseases in humans and accounts for almost 70% of all reported zoonotic cases. Broiler meat is considered the main source of campylobacteriosis in humans (7). Food and Agriculture/World Health Organisations reports indicate that there is a linear relationship between the prevalence of campylobacter in flocks and the likelihood of campylobacteriosis in humans (8). Worldwide an average prevalence of campylobacter contamination on poultry carcasses has reported being in the range of 60 % to 80 % (9). Reduction of the prevalence of positive flocks would contribute substantially to the reduction of human diseases (8). Although antibiotics are generally used to kill bacteria, treatment with antibiotics cannot completely prevent campylobacteriosis. Furthermore, the increasing resistance of Campylobacter to antibiotics is a growing public health problem worldwide and can have a detrimental effect on human and animal health (10). Therefore, the search for ways to prevent Campylobacter in broiler houses continues, and to date a number of products, including essential oils and plant extracts, spices, organic acids, probiotics and prebiotics, have been recognised and proposed as antibiotic alternatives in farm animal nutrition (11). Probiotics have been defined as microorganisms used as feed additives that can have a positive

effect on the animal host and improve gut balance (12, 13). Following the ban on antibiotics in animal feed, probiotics have attracted increasing interest and are considered biological products that stimulate the immune system and provide defence factors against pathogenic bacteria. Furthermore, some studies have reported that probiotics can be as effective as antibiotics, especially under stressful conditions (11). Some researchers reported that probiotics reduced of intestinal *Salmonella* and Campylobacter colonization (11, 14). Recently, probiotics have become commercially available as feed additives, mainly Lactobacillus spp. strains and various species including Streptococcus thermophilus, Enterococcus faecium, Enterobactris faecalis, Bifidobacteria species, *Saccharomyces cerevisiae* and Toulopsis sphaerica (15). This study reviewed studies from the last 5 years in which probiotics were administered in broiler diets to investigate the effects on Campylobacter colonisation and interpreted the results.

Narrative

In this review, the Google scholar search engine was used to scan related research papers on the use of probiotics and their effects on *campylobacter* in broiler production. The keywords chosen were "*campylobacter*, probiotic, broiler" for the search and the date was limited to 2018 to 2022. A total of 500 articles on the fifty web pages were scanned and examined. Full-text, original research and English-language articles that included *in-vivo* experiments involving the administration of probiotics with feed or water to determine *Campylobacter* in broiler samples were selected for review. Dissertations, reviews, *in-vitro* experiments, field studies, and methodological studies were excluded from the review. A total of 11 original research articles were found in which different probiotic bacteria were used in *in-vivo* experiments with broiler chickens. The results concerning *Campylobacter* counts in the samples were reported according to the statistical data and the p-values of the articles were considered but, statistically insignificant numerical changes were not taken into account. The results were evaluated and summarised in Table 1. The results showed that different probiotic bacterial strains were used to test on *Campylobacter* in broilers, but *Lactobacillus spp.* and *Bacillus spp.* were mainly preferred. On the other hand, in the studies for the determination of *Campylobacter* discussed in this review, the samples were mostly taken from the intestinal contents of caeca and faeces of broilers. As can be seen in Table 1., five studies reported a reduction in *Campylobacter* in broiler samples when probiotics were administered. The other five studies found that the use of probiotics in broiler production had no effect on *Campylobacter* numbers in the samples. Only one study found an increase in *Campylobacter* in broiler samples when probiotics were used.

Table 1. showed that probiotic strains may be an effective factor in colonising the intestinal tract of broilers with *Campylobacter* and that *Bacillus spp.* (mixed Bacillus spp. mixture) were effective in reducing *Campylobacter* in two out of three studies. Previous studies approved that probiotic bacteria are able to inhibit the growth of pathogenic microflora in the gastrointestinal tract of birds (27). In this review, research has been limited with the last five years to consider the recent advances, however previous reviews investigated studies over a more comprehensive period and concluded that probiotics as anti-*campylobacter* agents (28). However, similar to this paper, previous comprehensive reviews have reported that studies using probiotics individually have produced heterogeneous results and the difference between the *in vitro* and *in vivo* results could be partly explained by the complex gut microbiota of birds, which might interact with anti-*Campylobacter* activity (29). On the other hand, in vitro studies have announced that probiotic bacteria are able to inhibit *Campylobacter spp.* (30) and, moreover, probiotics have been reported that affect motility as well as colonisation of *C. jejuni* under *in vivo* and *in vitro* conditions (31). The problem to approve probiotics' effects on broiler *campylobacter* is due to differences in methodological and application differences between experimental researches. A previous review (32) confirmed that differences between studies were due to experimental conditions, detection methods, differences within and between chicken flocks, and the complexity of probiotic-host interactions, and mentioned that these complex interactions led to difficulties in validating the subsequent effects of administering certain probiotic strains.

Table 1. Results of *in-vivo* studies that used probiotics to determine effects on *Campylobacter spp*. colonization in broiler

	Reference	Probiotic strain	*Campylobacter* strain	Samples	Result
1	(16)	*Lactobacillus reuteri* *P*ediococcus acidilactici *B*ifidobacterium animalis *E*nterococcus faecium	*Campylobacter coli*	Caeca Carcass	No effect
2	(17)	*Saccharomyces cerevisiae boulardii*	*Campylobacter Jejuni*	Faecal	Reduction
3	(18)	*Bacillus spp.*	*Campylobacter Jejuni*	Faecal	No effect
4	*(19)*	*Escherichia coli*	*Campylobacter Jejuni*	Caeca	Reduction
5	(20)	*Lactobacillus plantarum*	*Campylobacter Jejuni*	Faecal	No effect
6	(21)	*Bacillus spp.*	*Campylobacter Jejuni*	Caeca	Reduction
7	(22)	*Lactobacillus spp.*	*Campylobacter spp.*	Caeca	Increase
8	(23)	*Bacillus spp.*	*Campylobacter spp.*	Caeca	Reduction
9	(24)	*Bacillus licheniformis*	Campylobacter spp.	Caeca	No effect
10	(25)	*Bacillus amyloliquefaciens*	Campylobacter spp.	Caeca	No effect
11	(26)	*Lactobacillus casei*	*Campylobacter jejuni*	Cecum Ileum Jejunum	Reduction

Conclusion

Campylobacter, a zoonotic pathogen causing campylobacteriosis in humans and of public health importance, is directly linked to the consumption of poultry meat. Therefore, in order to reduce the *Campylobacter* load in poultry meat, there has been interested in new approaches, such as the use of probiotics as an alternative to antimicrobials in broiler production. Research on the use of probiotics in broiler production is considerable and most of them have focused on performance, pathogen control and immunological parameters. Recently, probiotic bacterial strains have been marketed individually or in mixed preparations as feed additives for broiler production. Despite a large number of studies on the effects of probiotics on *Campylobacter* colonisation in broiler production, there was no consensus on efficacy. This study examined five hundred articles published in the last five years and very few of them had tested the probiotic effects on campylobacter numeration in the broiler as *in-vivo*. The data from eleven studies showed that the results of the experimental *in- vivo* studies were not consistent to suggest that probiotics can significantly reduce campylobacter in broilers. While there is some evidence that probiotics can reduce the *Campylobacter* load in broiler flocks, it is clear that further repetitive studies on the use of probiotics in broiler diets are needed to determine effective strains, dosages, and delivery methods.

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P59 Impact of Low Levels of Ca and P in Feed on Broiler Performance, Ca and P Digestibility, Bone Strength and Ash

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Abstract

The trial demonstrated that lowering the Ca and P levels in the feed (when formulating with 1000 FTU/kg OptiPhos® Plus to obtain grower and finisher feed without added MCP) does not have a negative impact on broiler performance, increases Ca and P digestibility, and only has a limited impact on bone ash and bone strength.

Introduction

The primary source of Ca in broiler feed when using phytase to reduce inorganic P sources is limestone. Limestone however has some negative aspects on broiler performance. Besides the fact that it tends to reduce feed intake, it also acts as a buffering agent in the gizzard, compromising protein denaturation and thereby digestion. However, its most negative aspect might be the reduction in P digestibility, linked to the complexation of the phytate molecule and thereby limiting the physical accessibility of the molecule by phytase. In particular fine limestone, which shows high solubility seems to have a negative impact [1-5]. Therefore, it is of interest to learn to what level limestone (Ca) inclusion levels in the feed can be lowered without compromising performance and bone strength.

Materials and Methods

Three times 6 pens with 24 male Ross 308 male per pen were fed a feed containing a phytase (OptiPhos® Plus) at 1000 FTU/kg (1.76 g/kg available P (aP) matrix value) and an endo-1,4- beta-xylanase (Hostazym® X) was added on top (1500 EPU/kg; Table 1). Starter feed (day 1-10) contained 22.0 % crude protein (CP), 1.23 % dig Lys, 2925 kcal/kg ME broiler, 0.85 % Ca and 0.45 % available P (aP). Grower feed (day 10-21) contained 20.3 % CP, 1.11 % dig Lys, 3000 kcal/kg ME broiler, 0.70 % Ca and 0.36 % aP. The finisher feed (day 21-35) contained 18.7 % CP, 0.98 % dig Lys, 3050 kcal/kg ME broiler, 0.60 % Ca and 0.30 % aP. In addition, a second feeding strategy was initiated by reducing the Ca and aP level in all feeds with 2.0 g and 1.5 g, respectively so that the grower and finisher feed did not contain any added inorganic P (Table 2). Technical performance was measured for every feeding phase. At day 21, the tibiae from 3 birds per pen were removed and analysed for bone strength. Afterwards, the 3 tibiae were pooled into 1 sample for the determination of ash after fat extraction and drying (a total of 9 samples per treatment).

Results

The technical performance was very high (close to 2.7 kg bird weight at 35 days with an FCR of 1.4) demonstrating the merits of Hostazym® X and OptiPhos® Plus (Table 2). Lowering the Ca and P level in the feed did not have a negative effect on performance; on the contrary it increased end weight with 22 g (Fig. 1). However, it lowered the bone ash and bone strength slightly, although not significantly (Table 2). The bone ash and strength of the birds on the low Ca and P feed are still above the level known to cause leg disorders. Lowering Ca and P significantly increased the Ca and P digestibility (Table 2) and showed a significantly lower uptake of dig. Ca and an increased intake of dig. P (Table 2).

Table 1. Feed and nutrient composition (g/kg)

	Starter (d 1-10)		Grower (d 11-21)		Finisher (d 22-35)	
Feed composition (g/kg)	Normal Ca and P	Low Ca and P	Normal Ca and P	Low Ca and P	Normal Ca and P	Low Ca and P
Corn	386	399	415	428	446	458
Wheat	200	200	200	200	200	200
Soybean meal 49	310	308	260	258	215	213
Rapeseed meal	15	15	25	25	30	30
Sunflower meal	15	15	25	25	30	30
Soybean oil	33.0	28.5	41.5	37.0	46.5	42.5
Limestone 0.4mm	15.7	11.5	13.9	9.7	11.8	7.2
MCP	6.7	4.5	2.2	0.0	1.4	0.0
Others*	18.7	18.7	17.1	17.2	19.4	19.5
Calculated analysis (g/kg)						
ME (Kcal/kg)	2925	2925	3001	3001	3050	3050
Crude protein	219	219	203	203	187	187
Dig Lys	12.3	12.3	11.0	11.0	9.8	9.8
Ca	8.5	6.5	7.0	5.0	6.0	4.0
P total	5.3	4.8	4.3	3.8	3.9	3.7
aP	4.5	4.0	3.4	3.0	3.2	2.9

* including salt, $NaHCO_3$, synthetic amino acids, mineral and vitamine premix, Ti02 as marker

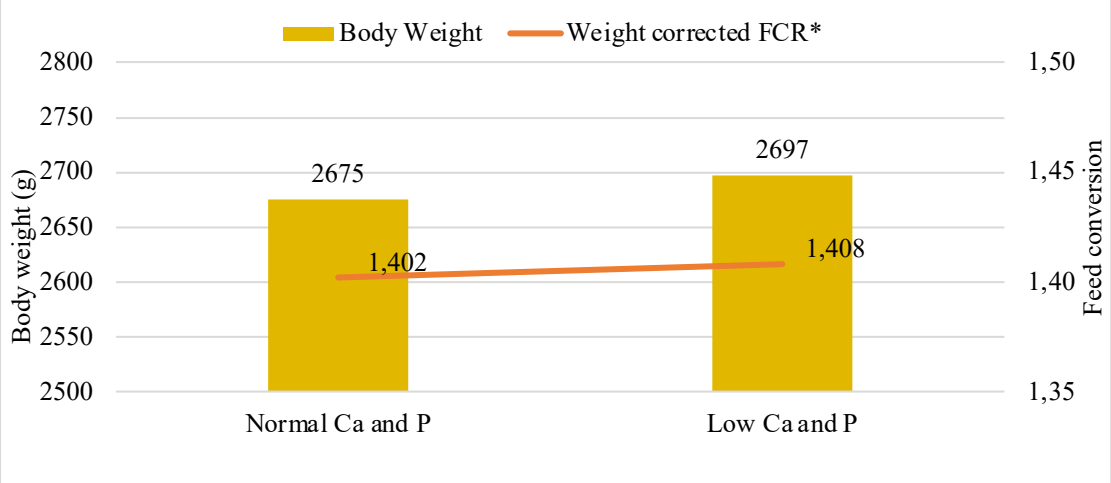

Figure 1. Effect on body weight and corrected feed conversion on day 35

Table 2. Effect on bone ash and bone strength (day 21) and Ca and P digestibility (day 35)

	Normal Ca and P	Low Ca and P
Bone ash (%)	47.5	46.8
Bone strenght (N)	548	532
Ca digestibility	51.7[a]	66.4[b]
P digestiblity	77.3[b]	83.2[a]
Dig Ca intake (g/day)*	0.62[a]	0.49[b]
Dig P intake (g/d)*	0.53	0.57

Conclusion

It can be concluded from this trial that Ca levels can be reduced substantially in broiler feed without loss of performance and increasing Ca and P digestibility while only limited impacting bone ash and bone strength. This indicated that the Ca requirements for optimal growth and optimal bone ash/strength are different, indicating that requirements are higher for bone ash/strength.

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P60 Investigation of the Effect of Sex and Fatty Acids on MDA Levels in Japanese Quail with Multiple Linear Regression Analysis

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Abstract

Fatty acid composition is an important indicator of disease prevention and food quality. Along with genetic advances, while productivity increases in poultry breeding, there is an increase in stress factors accordingly. Stress factors adversely affect meat quality and fatty acid composition by triggering oxidative stress. In this study, it was aimed to examine the effect of fatty acids on MDA with multiple linear regression analysis. As a result of the analysis, it was found that C14:1, C16:1, C18:0, C18:3 n3, C20:5 n3 and C22:6 n3 fatty acids were statistically significant ($p<0.05$), while C20:3 n3 fatty acids were nearly significance level ($p=0.093$). The multiple explanatory coefficient (R^2) of the regression model was calculated as 0.480. As a result, the relationships between MDA and fatty acid composition were revealed using multiple linear regression analysis. In addition, it is thought that the regression model can be developed by establishing regression models with different variables or by increasing the number of independent variables in future studies.

Keywords: Fatty acid profile, Japanese quail, MDA, regression analysis

P61 The Relationship Between IGF-1, Myogenin and UCP Genes Expression Levels in Muscle Tissue and some Yield Characteristics in Japanese Quails

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Abstract

Japanese quail, which is one of the alternative animal product sources, gains importance in the poultry industry due to its short generation interval, high feed efficiency, relatively resistance to diseases, and ease of breeding. In this study, five groups were formed with Japanese quails fed with basal ration and in addition to basal ration, palmerosa oil, Lemon myrityl oil, α-tops and cyclodextrin sources. IGF-1 (Insulin-Like Growth Factor 1), Myogenin and UCP (Uncouple Protein) gene expression levels in muscle tissue were investigated in male quails. In addition, body weight, warm carcass and cold carcass weights were determined and the relationships of target genes with these parameters were investigated. It was determined that IGF-1 gene expression levels were upregulated approximately 2 and 11 folds in Lemon myrityl and Cyclodextrin groups, respectively ($P<0,05$). Compared to control UCP gene expression levels were upregulated approximately 2 folds in Lemon myrityl and α-tops groups ($P<0,05$). UCP with myogenin ($r=0.586$; $P<0.001$), slaughter body weight with warm carcass ($r=0.672$; $P<0.001$) and cold carcass ($r=0.731$; $P<0.001$) and warm carcass with cold carcass ($r=0.913$; $P<0.001$) parameters were found to have positive correlations. As a result, it was determined that there is a significant relationship between the ration content and IGF-1 and UCP in muscle tissue development in quails, and it may be important to consider the Myogenin gene along with these genes in associating the ration content with yield and quality parameters.

Keywords: Japanese quails, gene expression, IGF-1, Myogenin, UCP

P62 Validation of the Nutritional Matrix of a Multienzyme Complex Through Performance and Digestibility Improvement

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Abstract

When evaluating and comparing nutritional matrix values for feed enzymes, one should always carefully consider how these values have been determined and whether they are scientifically well founded. To further validate an existing nutritional matrix of a commercial multi-enzyme complex (xylanase + β-glucanase + cellulase + amylase + protease or MEC), the following study was initiated. 320 one-day-old male Ross 308 chickens were randomly assigned to 4 treatments, each replicated 8 times: T0, positive control without enzymes (PC); T1, negative control (NC), PC reformulated to reduce apparent metabolizable energy (AME), digestible amino acids (AA) and crude protein (CP) by 65 kcal/kg and 2% respectively; T2, NC + MEC at 250 g/ton; T3, NC + a commercial xylanase and β-glucanase complex (XBC) at 50 g/ton. The total treatment duration was 35 days, with a 3-phase feeding system (starter 1-11d), grower (12-24d) and finisher (25-35d). Performance parameters were recorded per feeding phase and for the overall trial. Digestibility was determined during the grower period from d18 to d21. For the whole experimental period, no statistical difference in feed conversion ratio was found between PC (T0) and NC + MEC (T2) birds, while that of the NC (T1) and NC + XBC birds was significantly worse ($P<0.001$) compared to PC (T0) and NC + MEC birds (T2). Birds fed NC diets supplemented with MEC (T2) were characterized by the highest starch total tract digestibility ($P<0.0001$) while birds fed NC diets (T1) were characterized by the lowest. Total tract digestibility of Gross Energy (GE) was significantly better ($P<0.0001$) in birds from PC (T0) and NC + MEC (T2) compared to the NC (T1). The same effect was observed for the AME/GE ratio. AMEn of diets fed to PC (T0) and NC + MEC birds (T2) was not significantly different despite of the gap created by MEC nutritional matrix. AMEn of the diet fed to NC (T1) and NC + XBC birds (T3) was not different, and it was lower compared to PC (T0) and NC + MEC birds (T2), which is also consistent with the FCR results. When adding the MEC at 250 g/ton of feed in reformulation (reducing AME, digestible AA and CP by 65 kcal/kg and 2% respectively) feed cost could be reduced by 12 €/t of feed (on average) compared to the PC diet, without compromising broiler performance. The XBC did not show the ability to recover the nutritional gap following reformulation.

Keywords: Multi-enzyme complex, nutritional matrix, broiler performance, digestibility

Introduction

It is well known that feed represents the most important cost in poultry production. Together with variable costs, it can reach up to 70% of the total cost in a poultry operation [1]. Improving feed efficiency, therefore is one of the main production targets of every poultry producer. Enzymes are commonly used to optimize feed cost and, amongst them, carbohydrases, are extensively used in monogastric nutrition [2]. Feed enzymes should have a proven, well-researched nutritional matrix assigned to them, to ensure the most cost-effective feed reformulation. Hence when evaluating nutritional matrices between feed enzymes, poultry nutritionists should not only pay attention to the absolute figures but more importantly how these are substantiated. Ideally, they are obtained from a safely, corrected average improvement of nutrient digestibility, recorded in different poultry trials. This creates a trustful and reliable nutritional matrix to optimize feed cost without impairing poultry performance. The aim of the following study is to validate the proposed matrix value of a commercial multi-enzyme complex (MEC).

Material and Methods

320 one-day-old male Ross 308 chickens (average initial weight 41.2 g) obtained from a commercial hatchery were randomly assigned to 4 treatments with 8 replicates (pens) of 10 birds each:

- T0: positive control (PC) without enzymes.

- T1: negative control (NC), PC reformulated to reduce apparent metabolizable energy, digestible amino acids and crude protein by 65 kcal/kg and 2% respectively (reduced the feed cost by 12 €/tonne compared to the PC)
- T2: NC + MEC (xylanase + β-glucanase + cellulase + amylase + protease) at 250 g/ton.
- T3: NC + a commercial xylanase + β-glucanase complex (XBC) at 50 g/ton.

The experiment lasted for 35 days and was divided into 3 feeding periods: (starter 1-11d), grower (12-24d) and finisher (25-35d). Diets were formulated to meet or exceed FEDNA 2018 nutritional recommendations for broiler chickens [3]. The composition of experimental diets and calculated values are presented in Table 1. Average pen weight and feed intake were recorded at the end of every feeding phase. Feed conversion, daily growth rate, bird-days and daily feed intake per bird was calculated per feeding phase and for the overall trial. Digestibility was determined during the grower period from day 18 to 21, using titanium oxide as indigestible marker. Collection trays were installed in every floor pen on day 18 to 21, for collecting one excreta sample per pen, in total eight excreta samples per treatment,.

Results and Discussion

Performance :The effect of supplementing different enzyme preparations to broiler diets on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) is presented in Table 2.

Digestibility : The effect of supplementing different enzyme preparations to broiler diets on nutrient retention and total tract digestibility is presented in Table 3.

There were significant differences ($P=0.0001$) in dry matter and nitrogen retention. Birds from PC treatment (T0) were characterized by the highest DM retention, while NC + MEC birds (T2) showed higher dry matter retention compared to NC (T1). Nitrogen retention of the NC birds (T1) was the lowest compared to all other treatments ($P<0.05$). There were no differences in total tract crude fat digestibility. NC + MEC birds (T2) were characterized by the highest starch total tract digestibility ($P<0.05$) while birds from negative control treatment (T1) were characterized by the lowest. Total tract digestibility of GE was significantly better ($P<0.05$) in birds from PC (T0) and from NC + MEC (T2) compared to the NC (T1).

AMEn of the diets fed to the PC (T0) and NC + MEC birds (T2) was not significantly different, despite of the gap created by reformulation with the proposed nutritional matrix. This effect is consistent with the lack of significant differences in FCR and BWG. AMEn of the diet fed to the NC (T1) and NC + XBC birds (T3) was neither significantly different, however that of the NC birds was lower ($P<0.05$) compared to the PC (T0) and NC + MEC birds (T2), which is also consistent with the FCR results (Figure 2).

The results of the present study confirm the previous findings, that a MEC contributes to improving growth and FCR when added to broiler diets [4, 5]. NSP-degrading enzymes (NSPases) are commonly added to poultry diets to counteract the potential anti-nutritional effects caused by non-starch polysaccharides [6, 7]. In the early life of the chicken, its digestive capacity is not yet fully developed. According to Noy and Sklan [8] net duodenal secretion of amylase, trypsin, and lipase is low at 4 days of age and increases 100-, 50-, and 20-fold, respectively, by day 21. A MEC containing NSP degrading enzymes, together with an amylase and protease can increase the digestive capacity of the chicken: by supplying multiple NSPases to attack different portions of the cell wall at once, and by providing other hydrolases such as protease and alpha amylase, which enhances the action of the endogenous digestive enzymes thus ensuring the most efficient energy and amino acid release from the feed.

Table 1. Composition of experimental basal diets and calculated nutritional value

Ingredients (%)	Starter (1-11d)		Grower (12-24d)		Finisher (25-35d)	
	PC	NC	PC	NC	PC	NC
Maize	43.10	26.08	45.11	27.77	50.23	32.86
SBM (45% CP)	39.13	34.33	36.82	31.53	31.61	27.37
Wheat	10.00	20.00	10.00	20.00	10.00	20.00
Triticale	-	10.00	-	10.00	-	10.00
Sunflower Meal	-	2.28	-	3.00	-	2.00
Soybean oil	3.42	2.88	4.24	3.81	4.71	4.25
Limestone	1.40	1.42	1.27	1.28	1.13	1.15
MCP	0.94	0.91	0.76	0.73	0.62	0.60
NaCl	0.26	0.25	0.25	0.24	0.23	0.23
Sodium Bic.	0.12	0.13	0.06	0.07	0.05	0.05
HCl-Lys	0.20	0.28	0.13	0.20	0.12	0.18
DL-Met	0.33	0.32	0.30	0.28	0.24	0.23
L-Thr	0.10	0.12	0.05	0.08	0.04	0.07
Premix (vit/min)[1]	1.00	1.00	1.00	1.00	1.00	1.00
Phytase 5000[2]	0.01	0.01	0.01	0.01	0.01	0.01
Nutrients (%)						
Crude Protein	22.50	22.15	21.50	21.15	19.50	19.15
Crude Fat	5.74	4.81	6.59	5.75	7.13	6.28
Crude Fiber	3.19	3.45	3.12	3.49	2.98	3.30
Ca	1.00	4.00	0.91	0.91	0.82	0.82
dig P	0.49	0.49	0.45	0.45	0.41	0.41
Na	0.18	0.18	0.16	0.16	0.15	0.15
Cl	0.24	0.25	0.22	0.23	0.21	0.22
DigLYS	1.24	1.22	1.126	1.100	1.000	0.980
DigMET	0.618	0.598	0.579	0.551	0.500	0.480
DigMET+CIS	0.910	0.890	0.862	0.833	0.760	0.740
DigTHR	0.800	0.788	0.730	0.718	0.650	0.638
DigTRP	0.244	0.236	0.232	0.224	0.207	0.20
DigVAL	0.912	0.877	0.874	0.84	0.790	0.756
DigILE	0.851	0.811	0.813	0.772	0.727	0.690
DigARG	1.376	1.317	1.312	1.256	1.170	1.111
AME (Kcal/kg)	2950	2885	3035	2970	3125	3060
Feed cost (€/ton)	**481.1**	**470.2**	**481.7**	**471.1**	**474.0**	**465.0**

[1]mineral and vitamin premix provides per kg diet: IU: vit. A 11250, cholecalciferol 2500; mg: vit. E 80, menadione 2.50, vit. B12 0.02, folic acid 1.17, choline 379, D-pantothenic acid 12.5, riboflavin 7.0, niacin 41.67, thiamin 2.17, D-biotin 0.18, pyridoxine 4.0, ethoxyquin 0.09, Mn 73, Zn 55, Fe 45, Cu 20, I 0.62, Se 0.3. [2]phytase 5000 provides 0.15% dig P, 0.165% Ca and 0.035 Na.

Table 2. Growth performance of broiler chickens fed different diets supplemented with different enzymes

	Starter (1-11 d)			Grower (12-24 d)			Finisher (25-35 d)			Total (1-35 d)			
	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR	FCR_a*
T0	234	269	1.152	837^{ab}	1531^{a}	1.830^{a}	1106	1519^{ab}	1.381^{a}	2177^{b}	3319^{a}	1.526^{a}	1.531
T1	223	266	1.190	812^{b}	1600^{b}	1.976^{b}	1054	1753^{c}	1.666^{c}	2089^{a}	3618^{c}	1.732^{c}	1.757
T2	225	266	1.183	829^{ab}	1552^{a}	1.877^{a}	1092	1507^{a}	1.380^{a}	2146^{ab}	3325^{a}	1.549^{a}	1.561
T3	228	274	1.200	866^{a}	1599^{b}	1.850^{a}	1039	1596^{b}	1.539^{b}	2132^{ab}	3468^{b}	1.627^{b}	1.642
P	0.358	0.68	0.253	0.142	0.0067	0.0020	0.164	<0.01	<0.001	0.087	0.001	0.001	-

T0: positive control (PC) without enzymes; T1: negative control (NC), PC reformulated to reduce apparent metabolizable energy, digestible aminoacids and crude protein by 65 kcal/kg and 2% respectively T2: NC + MEC (xylanase + betaglucanase + cellulase + amylase + protease) at 250 g/ton; T3: NC + a commercial xylanase + betaglucanase complex (XBC) at 50 g/ton

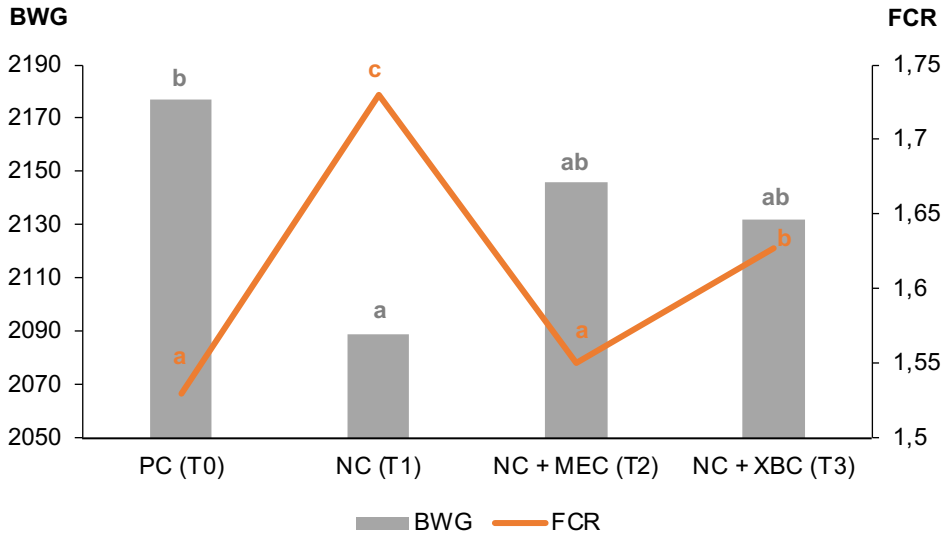
BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

*FCR_a is adjusted FCR at 2.2 kg BW calculated per Ross 308 Management Handbook 2014.

a–c Means without a common superscript differ significantly at P < 0.05.

For the whole experimental period (1-35 d), no statistical difference was found between PC (T0) and NC + MEC (T2) birds for BWG, FI or FCR, whereas FCR of NC (T1) and NC + XBC birds was significantly worse (P<0.05) compared to PC (T0) and NC + MEC birds (T2). Overall feed intake was the highest (P<0.05) for NC birds (T1) and NC + XBC birds (T3). There were no differences in growth performance during starter period (1-11 d). During the grower period (12-24 d), the highest feed intake (P<0.05) and worst FCR (P<0.05) was recorded for NC birds (T1). During the finisher period (25-35 d) the worst FCR (P<0.05) was recorded for both NC (T1) and NC + XBC birds (T3).

Figure 1. Overall trial BWG (g) and FCR



a–c Means without a common superscript differ significantly at P < 0.05.

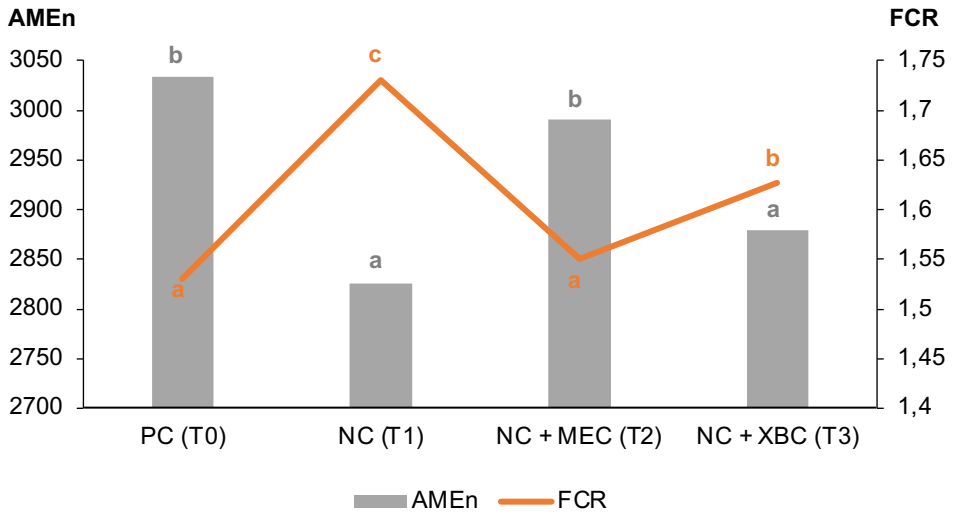
Table 3. Digestibility and retention of nutrients from 18 to 21d of ag

	Retention		Total tract digestibility			
	Dry matter	Nitrogen	Crude Fat	Starch	Gross Energy	AMEn(kcal/kg)
T0	70.9^{c}	62.9^{c}	79.6	95.4^{b}	73.4^{c}	3034^{b}
T1	67.0^{a}	56.8^{a}	77.8	93.2^{a}	68.9^{a}	2826^{a}
T2	68.8^{b}	60.8^{bc}	80.6	97.0^{c}	71.6^{b}	2991^{b}
T3	68.1^{ab}	60.2^{b}	79.2	95.7^{b}	70.4^{ab}	2879^{a}
P-value	0.0001	0.0002	0.396	<0.0001	<0.0001	<0.0001

T0: positive control (PC) without enzymes; T1: negative control (NC), PC reformulated to reduce apparent metabolizable

energy, digestible amino acids and crude protein by 65 kcal/kg and 2% respectively T2: NC + MEC (xylanase + beta-glucanase + cellulase + amylase + protease) at 250 g/ton; T3: NC + a commercial xylanase + beta-glucanase complex (XBC) at 50 g/tonne a–c Means without a common superscript differ significantly at $P < 0.05$.

Figure 2. Overall trial FCR and AMEn



a–c Means without a common superscript differ significantly at $P < 0.05$.

Conclusions

This trial demonstrates, including a commercial complex of a xylanase, β-glucanase, cellulase, amylase and a protease at 250 g/tonne of broiler feed in a reformulation (reducing apparent metabolizable energy, digestible aminoacids and crude protein by 65 kcal/kg and 2% respectively) can reduce feed cost by 10 €/t of feed, compared to a non-supplemented, non-reformulated standard diet, without compromising bird performance. The commercial blend with only a xylanase and β-glucanase did not show the ability to recover the nutritional gap following reformulation.

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P63 The Need for Chromium Supplementation in Poultry: A Review

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Abstract

Over the past few decades sporadically research has been done into the benefits of chromium in livestock diets, primarily in pig and ruminant diets [1]. However, it's only in recent years that scientific interest in exploring the benefits of chromium in poultry has considerably increased and supplementation in feed is gaining momentum. Because of its non-historical use, chromium was subjected to approval from the Food and Drug Administration (FDA) in the USA and The European Food Safety Authority in Europe (EFSA). Currently, three organic chromium sources are approved for animal application by the FDA: chromium propionate, chromium picolinate and chromium yeast. In summary, chromium supplementation has been shown to improve growth performance of livestock, to support animal performance during heat stress, to promote a better carcass quality and enhance the immune response to vaccination.

Introduction

The intensification of poultry production has resulted in the continual search for nutritional compounds that can optimize production yields. Nutrition remains one of the most important factors in supporting performance and carcass quality, as even with flawless genetics production targets will not be met, unless an optimal diet is provided. Trace minerals e.g play a vital role in various metabolic, enzymatic and biochemical functions, all of which can influence bird performance, health and carcass traits [2]. Chromium is one of has the potential to positively impact growth and reproductive performance, health benefits, immune response, carcass quality, egg production and quality traits (figure 1).

Chromium presents in many oxidation forms, among them trivalent (Cr3+) and hexavalent (Cr6+) are the most common. The hexavalent form is a highly toxic metal that acts as an epithelial irritant and is a known mutagen and carcinogen [3]. The trivalent form on the other hand, is increasingly identified as an essential nutrient in livestock, potentially bringing a variety of benefits to animals [4,5]. Several varieties of chromium, including chromium propionate (Cr Prop), chromium picolinate (Cr Pic), chromium chloride (Cr Cl), chromium methionine (Cr Meth) chromium histidine (Cr his), and chromium yeast have been assessed in livestock research, demonstrating substantial variability in stability, bioavailability and observed effects between sources.

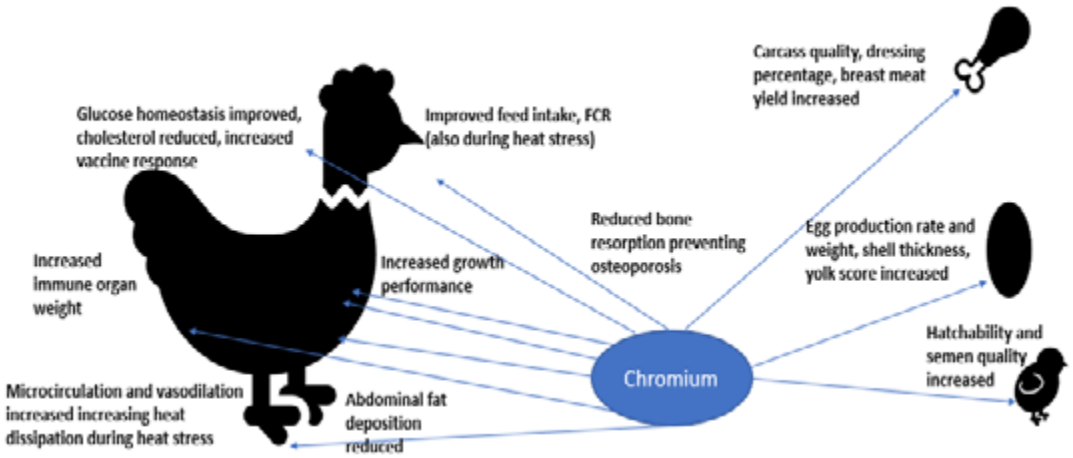

Figure 1. The reported benefits of chromium

Narrative

The effect of chromium on performance : Carbohydrates are used in animal feed as an energy source. Birds convert these carbohydrates into glucose which is ultimately released into the bloodstream. To reduce the glucose levels the pancreas releases insulin which together with the glucose enters the tissues to reach the animal cells. Chromium plays a prime role in the regulation of blood glucose levels in the bird's body. Absorbed dietary chromium is used for the synthesis of Glucose Tolerance Factor, a physiological enhancer of insulin activity, which binds to insulin and thus triples its activity [7]. Chromium is also vital for the synthesis of a specific low-molecular-weight chromium-binding protein which, upon conversion to chromodulin, activates the insulin signalling pathway. Insulin activates glucose transporters in the cell membranes so that the circulating glucose can enter the cells. Chromium optimizes the activation of these transporters. This results into a higher cell permeability which in turn increases the metabolism of carbohydrates, lipids and proteins [8]. Furthermore, Chromium is thought to influence the metabolism of triglycerides and cholesterol, although the exact mode of action for this is still unclear [8]. This is supported by the work of Debski et al. [9] that demonstrates that dietary Cr3+ reduces total blood cholesterol, low density lipoprotein cholesterol, triglycerides and non-esterified fatty acid serum concentrations. These metabolic interactions are thought to be the mechanism responsible for increased growth performance. Supplemental chromium has also been suggested to increase secretion of digestive enzymes due to chromium increasing microcirculation to the liver and pancreas and thereby improving function [10].

The effect of chromium during heat stress : Heat stress is an annual recurring challenge for the broiler industry, especially in warmer climates. The performance loss associated with heat stress, costs the industry a significant amount of loss in profitability. The mechanism here is related to the activation of the hormonal system during heat stress exposure and the subsequent secretion of corticosteroids into the blood stream [5, 11]. This in turns reduces insulin sensitivity and causes instability in glucose homeostasis [12]. Chromium supplementation, through its well described role in increasing insulin sensitivity and maintaining glucose homeostasis has been widely reported to mitigate the negative effects of heat stress [4,5].

Sahin et al. [13] investigated the effect of supplementing broilers exposed to heat stress with two different sources of chromium. The authors used 1200, 10-day-old Ross 308 broilers divided into 6 treatments (2X environmental temperatures and 3X diets – control, chromium (Chromium picolinate or Chromium histidine) 200µg/kg). Birds were either kept at thermoneutral temperatures or 34ºC for 8 hours a day until 42 days of age. The results of the study showed that both chromium sources partially alleviated the negative effects of heat stress seen in the heat-stressed control treatment, however, CrHis delivered a significant improvement in performance compared to the control. The results of this study agree with the findings of Hajializadeh et al. [14]. This study used 8 treatments: Control (standard diet, not heat stressed), Control + heat stress, Cr pic 500ppb+ heat stress, Cr pic 1000ppb + heat stress, Cr pic 1500ppb + Heat stress, Cr pic nano 500ppb + heat stress, Cr pic nano 1000ppb + heat stress and Cr pic nano 1500ppb + heat stress. Heat stressed birds were continually exposed to temperatures of 36°C. The results of the study revealed that supplementation of both Cr pic and Cr pic nanoparticles significantly improved the performance (BWG and FCR) of heat stressed chickens compared to the heat stress control group. Vignale et al. [15] demonstrated this effect when supplementing broilers exposed to cyclic heat stress with 200ppb of Cr Prop. Over a 60-day study these authors were able to show significantly increased body weights and reduced FCR compared to the non-treatment control. Likewise, in a second study the same authors showed that supplementing male broilers with 200 ppb of Cr Prop resulted in significantly increased body weights after 60 days compared to the control, as well as an 8-point reduction in FCR.

Chromium has also been shown to reduce rectal temperature and respiration rate during heat stress, likely linked to an improvement in microcirculation and vasodilation which facilitates heat dissipation [16, 17].

The effect of chromium on laying hens : Both egg production and egg quality have been shown to be beneficially affected by supplemental chromium. Lien et al. [18] reported that adding 800 ppb Cr Pic in the diet resulted in significantly increased egg production compared to non-supplemented hens. Liu et al [19] found that 100 ppb chromium chloride resulted in significantly increased egg weight, egg number and lay percentage. Sahin et al. [20] found that supplementing quails with 200, 400, 800, or 1200 ppb chromium resulted in a linear increase in shell thickness, egg specific gravity and Haugh unit score in line with increasing dose. There is also some evidence that chromium may help improve bone health by preventing osteoporosis. Human studies show that chromium supplementation reduces bone resorption in post-menopausal women. Therefore, chromium supplementation may be beneficial in prolonging the length of lay in laying hens by preventing osteoporosis and improving bone health [21].

The effect of chromium on carcass quality : Chromium supplementation can also improve the carcass quality of broilers. Rajalekshmi et al, [22] investigated the effect of Cr prop (0, 100, 200 and 400 ppb) on broilers. The study showed linear increases in carcass and breast meat yield in line with the chromium dose. This agrees with the work of Van Hoeck et al.

[6] who reported significant increases in dressing percentage, carcass yield, breast meat yield and a significant decrease in abdominal fat deposition with doses of both 200 and 400 ppb of Cr Prop compared to a non-supplemented control. Likewise, Lester et al. [23] found significantly improved carcass yield and breast weight when supplementing broilers with Cr Prop at 200 ppb.

Sahin et al. [24] report that even under heat stress conditions, the 400 ppb of Cr Pic significantly increased the cold carcass percentage compared to the control. Furthermore, Huang et al. [25] found that Cr Prop (0.4 or 2 mg) significantly reduced abdominal fat deposition, decreased cooking loss percentage and increased dressing percentage of broilers exposed to heat stress.

The effect of chromium on immune function **:** Chromium supplementation has also been reported to support the birds' immune function by enhancement of the cell mediated and humoral immune responses. Research has shown an increase in the weight of immune organs such as the spleen, bursa and thymus [26]. Rajalekshmi et al. [22] report that supplemental chromium increases lymphocyte proliferation and improves the heterophil/lymphocyte ratio.

Chromium supplementation has also been shown to support vaccine responses. Hajializadeh et al. [15] investigated the effects of Cr pic in a 42-day broiler study. The authors investigated the effect of Cr pic and Cr pic nanoparticles on the performance of broilers exposed to heat stress and their response to vaccination for Newcastle's disease and avian influenza. The study used 8 treatments: Control (standard diet, not heat stressed), Control + heat stress, Cr pic 500 ppb+ heat stress, Cr pic 1000 ppb + heat stress, Cr pic 1500 ppb + Heat stress, Cr pic nano 500 ppb + heat stress, Cr pic nano 1000 ppb + heat stress and Cr pic nano 1500 ppb + heat stress. Heat stressed birds were continually exposed to temperatures of 36°C and antibody titres were measured from blood samples taken at 21 and 42 days of age. The results of this study revealed that supplementation of both Cr pic and Cr pic nanoparticles significantly improved the performance (BWG and FCR) of heat stressed chickens (compared to the heat stress control group). Antibody titres for both avian influenza and Newcastle's disease were significantly higher in the groups supplemented with Cr pic and Cr pic nanoparticles compared to the control and control + heat stress groups. This was confirmed in the work of Uyanik et al. [26], which reported that when broilers were supplemented with 2.2, 4.5, 20, 40 and 80 mg/kg of Cr chloride for 44 days, antibody titers for IgG and IgM were increased.

The effect of chromium on reproductive traits **:** The effect of chromium on reproductive performance has not been well studied yet, however from the existing literature there is evidence to support the efficacy of supplemental chromium in increasing semen quality of birds. Abdallah et al. [28] investigated the effect of Cr Pic on the reproductive performance of Montazah chickens. The study concluded that 800 ppb of Cr Pic significantly improved semen quality (ejaculate volume, advanced motility and live sperm %), reproductive organ weights (ovary and tests) compared to non-supplemented birds, which subsequently resulted in also a significant higher hatchability. Improved hatchability has also been reported by Contreras et al. [29, 30] when supplementing quail with chromium methionine at 200 and 400 ppb respectively. The positive impact on semen quality could be attributed to the antioxidant activity of chromium which supports the integrity of the cell membrane and reduces the oxidants damage. Long and Kramer [31] confirmed that the reduction in malondialdehyde concentration is a marker to the integrity degree of sperm membranes and their ability of fertilizing.

Conclusions

The ongoing intensification of poultry production requires a constant attention to optimizing nutrition to allow broilers and layers to meet their genetic potential. The use of chromium has been demonstrated not only improve growth performance, but also to mitigate a number of commercial challenges experienced regularly in the industry such as heat stress, reproductive performance, protein quality and immune response. As a single solution, chromium supplementation offers significant value for money due to its ability to address numerous aspects of poultry production.

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Patentli, Tablet Formunda Enfeksiyöz Bronşitis Variant 2, Var 2-06 Suşu

Sadece Hayvan Sağlığında Kullanılır
Canlı Virüs Aşı Tabletleri
Enfeksiyöz Bronşitis Variant 2, Var 2-06 SUŞU

Tanımı: Aşı, SPF sürülerden gelen fertil yumurtalarda üretilmiş canlı attenue Enfeksiyöz Bronşitis virüsü içermektedir. Üretimi ve testleri, uluslararası standartlara uygundur. Canlı attenue Enfeksiyöz Bronşitis virüsü, dondurularak kurutulmuş, tabletlenmiş ve blisterlerde ambalajlanmıştır. IB virüsü variant 2 serotipi, Var2-06 suşu etkin ve güvenli kabul edilmektedir. **Farmasötik Formu:** Süspansiyonluk efervesan tablet **Aktif Maddelerin Adı ve Gücü:** Enfeksiyöz Bronşitis virüsü variant 2 serotipi, Var2-06 suşu 1 doz en az $10^{3.0}$-$10^{4.4}$ EID_{50} virüs içerir. **Endikasyonları:** Enfeksiyoz bronşitis virüsünün varyant 2 suşlarının neden olduğu solunum sistemi enfeksiyonları ile ilgili sıliar aktivite üzerindeki zararlı etkileri, solunum sistemi semptomları ve lezyonlarını azaltmak üzere, broyler, yumurtacı ve damızlık tavukların aktif immunizasyonu için kullanılır. Bağışıklığın başlangıcı: Aşılama tarihinden 21 gün sonra İmmunitenin süresi: İkinci aşılamadan sonra 7 hafta 1 günlük yaştan itibaren broyler ve yumurtacılarda güvenle kullanılabilir. **Hedef Türler:** Tavuklar (Broyler, Yumurtacılar, Damızlıklar) **Uygulama Metodu ve Yolu:** 1 günlük yaştan itibaren iri damlacıklı sprey aracılığıyla her tavuk başına bir doz uygulanır. 12 günlükken tekrar aşılama önerilir. **Kuluçkahanede iri damlacıklı spreyleme:** Kuluçkahanede spreyleme bir el püskürtücüsüyle veya sprey kabini ile uygulanabilir. • 100-300 mikron hassasiğında püskürtme başlığı (nozul) kullanınız • Dezenfektan içermeyen içme suyu veya steril su kullanınız • Su pH aralığı 5,5-7,5 olmalıdır • Su sıcaklığı 4°C -10°C olmalıdır, sprey esnasında soğuk tutmak için buz paketleri kullanınız • İzlemeyi kolaylaştırmak için gıdada kullanılabilir renklendirici ekleyiniz • Püskürtücüyü iyice yıkayınız ve herhangi bir dezenfektan kalıntısı içermediğinden emin olunuz • Gözlerinizi ve yüzünü korumak için uzun kollu giysi giyiniz ve maske takınız • Püskürtücü veya sprey kabinini temiz bir kağıtla kaplanmış boş bir civciv kutusuna püskürterek kalibre ediniz ve aşağıdakileri kontrol ediniz: o Uygulanan su miktarı, 100 civcivlik kutu başına 15-25 ml olmalıdır o Püskürtme başlığında kaçak olup olmadığını kontrol ediniz o Spreyin homojen olup olmadığını kontrol ediniz (izleme için suya gıdada kullanılabilir renklendirici eklenmesi şiddetle tavsiye edilir) • 120 dakikadan daha fazla dayanacak kadar yeterli miktarda aşı hazırlayınız • Aşı çözeltisini kaptan püskürtücüye ve sprey kabinine dökünüz • Toplam su hacminin, aşılanacak tüm civcivler için yeterli olduğundan emin olunuz *Kuluçkahanede uygulama:* • Tüm civcivlerin maruz kaldığından emin olunuz • Her civciv için tam bir doz kullanınız • Civcivler spreylemeden ıslak tüylerle çıkmalıdır • Sprey kabini kullanılıyorsa - işlemin daha iyi kontrolü için püskürtücüye iyi eğitilmiş bir personel yerleştiriniz • Aynı talimatlar manuel püskürtme için de geçerlidir • Ekipman kalibrasyonu, kuluçka ve püskürtme işleminin başında ve bir kez de ortasında yapılmalıdır *Bir günlük civcivler tavuk kümesine vardıklarında uygulama:* • Küməste spreyleme sırasında, ısıtıcıları ve fanları kapatınız • Civciv kutularını birbirine yakın yerleştiriniz • Tüm civcivlerin başları ve sırtları üzerine eşit miktarda püskürtünüz **Kümeste iri damlacıklı spreyleme:** Bu iş için belirlenmiş eğitimli bir kişinin olması zorunludur • Püskürtücüyü iyice yıkayınız ve herhangi bir dezenfektan kalıntısı bulunmadığından emin olunuz • Gözlerinizi, yüzünüzü ve cildinizi korumak için uzun kollu giysi giyiniz ve maske takınız • 100-300 mikron çapında damlacıklar üreten püskürme başlığına sahip, sabit basınçlı bir püskürtücü kullanınız. • Dezenfektan içermeyen içme suyu veya steril su kullanınız • Su pH aralığı 5.5-7.5 olmalıdır • Su sıcaklığı 4°C -10°C olmalıdır, sprey esnasında soğuk tutmak için buz paketleri kullanınız • İzlemeyi kolaylaştırmak için gıdada kullanılabilir renklendirici ekleyiniz • Püskürtme sırasında başlıktan sızıntı olmadığından emin olunuz • Püskürtme sırasında kullanılan miktarı gözlemleyebilmek için şeffaf bir kap kullanılması önerilir • Sprey uygulamasını temiz bir yüzey üzerinde kalibre ediniz (renklendirici kullanınız) • 1000 tavuk / doz başına su hacmi 250-300 ml olmalıdır • Püskürtme işleminden önce ısıtıcıları ve havalandırmayı kapatınız *Kümeste iri damlacıklı sprey uygulama:* • Aşı solüsyonunu dikkatlice püskürtme kabına dökünüz • Buz paketleri püskürtme esnasında aşıyı soğuk tutmak için kullanılabilir • Tavukları gruplara ayırınız ve hemen püskürtme işlemine başlayınız. Kümesteki her grupta yukarı ve aşağı iki kez, sabit bir hızda hareket ediniz. Püskürtme sırasında aşının boşalma süresi ve yürüme hızı ile ilişkili olarak deneyim kazanmak amacıyla önceden su ile uygulama yapılması önerilir • Tavukların kafalarının 30-50 cm yukarısından püskürtünüz • Uygun püskürtme ıslak tüylere ve tavuklarını başlarını sallamalarına sebep olacaktır • Püskürtmeden sonra, ısıtıcıları ve havalandırma sistemini tekrar devreye almadan önce en az 5-10 dakika bekleyiniz. Püskürtme işlemi mümkün olduğunca hızlı olmalı ve 20 dakikayı aşmamalıdır **Kontrendikasyonları:** Yoktur. **Yan Etkiler:** Yoktur. **Gebelik, laktasyon ve yumurtlama periyodunda kullanımı:** Kanatlılarda yumurtlama döneminde ya da yumurtlama periyodunun başlangıcından önceki 4 hafta içerisinde kullanmayınız. **Yasal Arınma Süresi:** Sıfır gün **Ambalaj:** Tablet başına 500, 1000, 2000, 2500, 5000 ve 10.000 doz. Her bir blister 10 tablet içermektedir. Her kutu 1 blister şeridi içermektedir. **Saklama Koşulları:** Buzdolabında saklayınız (+2°C ile +8°C arasında) ve blister üzerinde bildirilen son kullanma tarihine kadar kullanınız. **Raf Ömrü:** 23 ay **Uyarılar:** 1. Aşılanacak kanatlıların sağlık durumu iyi olmalı ve aşırı kalabalık, aşırı sıcak ya da soğuğa maruz kalma, kötü beslenme gibi herhangi bir stres altında olmamalıdır. 2. Canlı aşılar, gün ışığına, sıcağa, dezenfektanlara ve deterjanlara karşı hassastır ve bunlara maruz bırakılmamalıdır. 3. Uygun dozaj uygulanmalı, doz düşürülerek uygulanmamalıdır. 4. Blisterin delinmiş bölümlerindeki tabletler kullanılmamalıdır. 5. Aşının kullanılmayan bölümleri başka bir gün kullanılmak üzere saklanmamalıdır. 6. Açılmış blisterler ve sulandırılmış aşı derhal kullanılmalıdır. **Uygulayıcının Alması Gereken Önlemler:** Aşı kullanıldıktan sonra, uygulayıcı, onaylı bir dezenfektan ile ellerini yıkamalı ve dezenfekte etmelidir. **Atık ve Kullanılmayan Ürünlerin İmhası:** Atık materyal kaynatma, yakma ya da yetkili makamlar tarafından kullanımı onaylanmış uygun bir dezenfektana batırma yoluyla imha edilmelidir. **Pazarlama İzin Sahibinin ve Üretim Yerinin Adı, Adresi: Üretim Yerinin Adı ve Adresi:** Abic Biological Laboratories Ltd. Western Industrial Zone, Beit Shemesh 99100, İsrail **Pazarlama İzin Sahibinin Adı ve Adresi:** Phibro Hayvan Sağlığı Ürünleri Sanayi ve Ticaret A.Ş. Atatürk Cd. Esin Sk. Yazgan İş Merkezi No:3/9 Kozyatağı 34734, İstanbul-Türkiye **Pazarlama İzin Tarihi: 03.01.2013 Prospektüsün son onaylandığı tarih: 19.03.2018**

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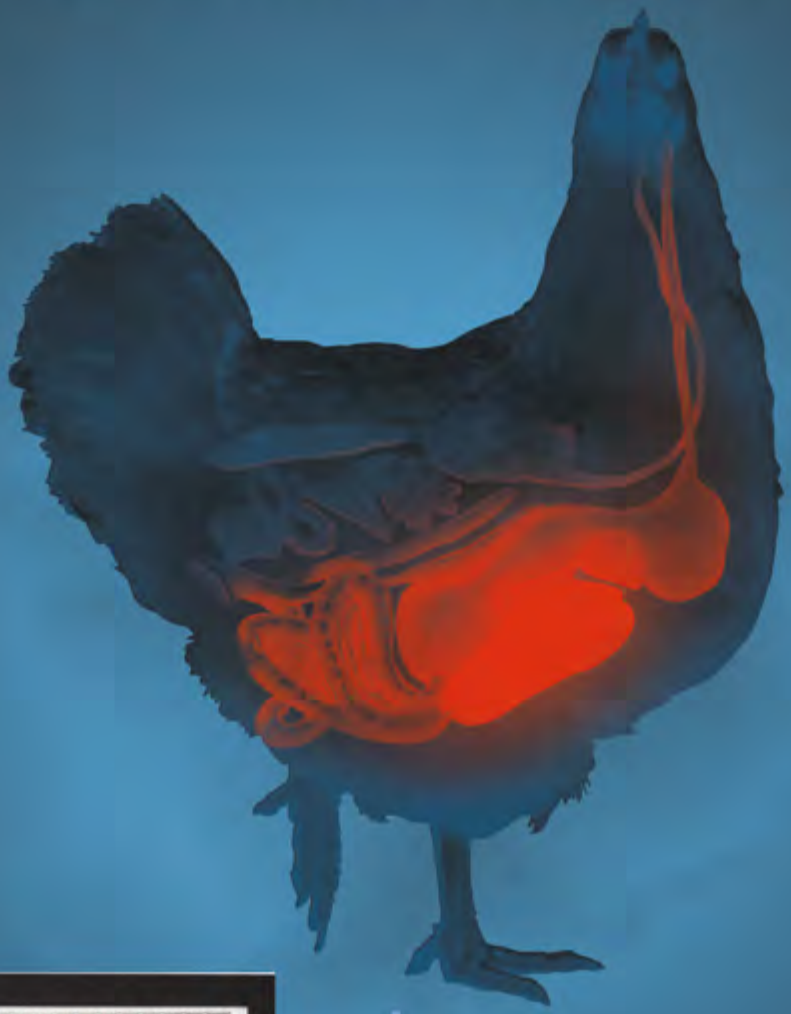


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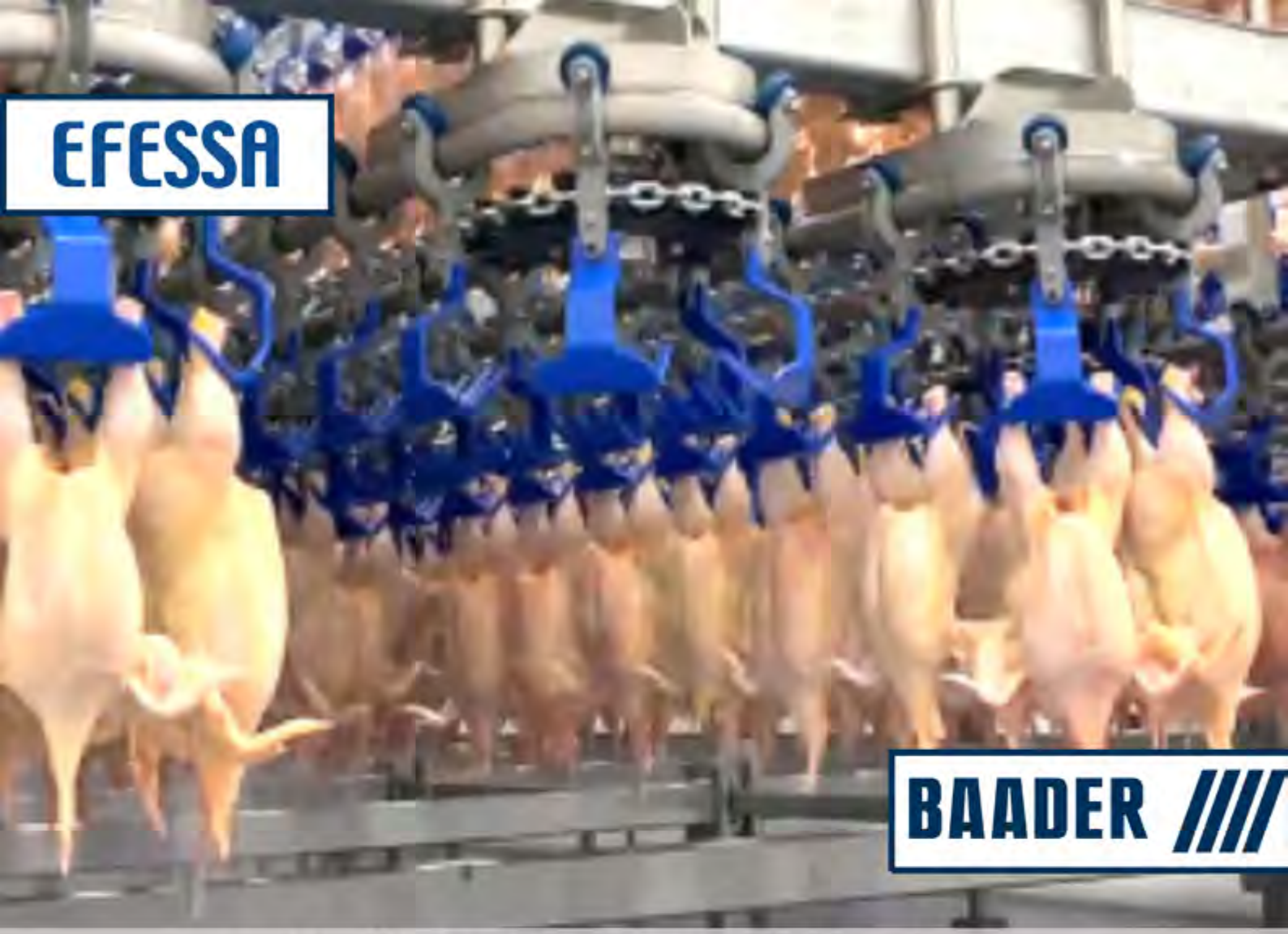

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