BESD-BİR
BEYAZ ET SANAYİCİLERİ VE
DAMIZLIKÇILARI BİRLİĞİ DERNEĞİ

SAĞLIKLI TAVUK
BİLGİ PLATFORMU

5th INTERNATIONAL POULTRY MEAT CONGRESS

24-28 April 2019 Starlight Convention Center & Sunrise Park Resort- ANTALYA- TURKEY

PROCEEDINGS

Photos

Submissions

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

BESD-BİR

BEYAZ ET SANAYİCİLERİ VE DAMIZLIKÇILARI BİRLİĞİ DERNEĞİ

BESD-BİR, provides approximately 91% of Turkey's total production of poultry meat, broiler hatching egg and day old broiler chick and represents the poultry sector on highest level.

The objective of the Association is to develop the poultry meat industry and to create values that would contribute to the establishments, to represent the sector most correctly and to establish the required communication between the government and the sector. 21 companies are the members of BESD-BİR. BESD-BİR cooperates with all stakeholders for the development of the sector.

Please visit the web site, http://www.besd-bir.org/ to have detailed information about Turkish Poultry Meat Producers and Breeders Association and send e-mail to besd-bir@besd-bir.org address for any of yours remarks, comments and questions.

BESD-BİR

BEYAZ ET SANAYİCİLERİ VE DAMIZLIKÇILARI BİRLİĞİ DERNEĞİ

OUR MEMBERS

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Healthy Chicken Information Platform (STBP)

Healthy Chicken Information Platform has been established in 2005 by the leading companies of the sector that realize 85% of poultry meat production of our country. The objective of the platform is to raise awareness of consumers and the society related with healthy poultry meat production and consumption, to emphasize the importance of poultry meat with regard to healthy diet and eliminate perception pollution and infollution due to unscientific arguments. Final target of the Platform is combine this value that has a significant role in healthy diet with the protein source and to contribute to raising enlightened, healthy, successful and happy generations.

Please visit the web site, http://www.sagliklitavuk.org/ to have detailed information about Healthy Chicken Information Platform and send e-mail to info@sagliklitavuk.org address for any of your remarks, comments and questions.

Wish you days full of health and with "chickens".

MEMBERS OF STBP

5th INTERNATIONAL POULTRY MEAT CONGRESS

PROCEEDINGS

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Editors

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(Ankara University)
Dr. Sait KOCA
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Starlight Convention Center & Sunrise Park Resort
Antalya / TURKEY

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Organization and Committees

Chair of the Congress:

Prof. Dr. Necmettin CEYLAN
Ankara University,Turkey

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Prof. Dr. Servet Yalçın *(Ege University, Turkey)*
Prof. Dr. Sevinç Yücecan *(Near East University, Turkish Republic of Northern Cyprus)*

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Dear Stakeholders of Poultry Meat Industry, Dear Friends

First four of the International Poultry Meat Congress which is traditionally organised by Turkish Poultry Meat Producers and Breeders Association (BESD-BİR) in each two years has realized in 2011, 2013, 2015, 2017 spring in Antalya with increased participation and success. The quality of the fifth congress has successfully reached to the international standards with it's high quality content including 19 keynote speakers and 49 short oral presentations in 22 scientific sessions distributed into 3 different meeting rooms and 54 poster presentations. We have around 1400 participants including family members and, almost 1000 active national and international participants from 32 countries registered in the present one which has significant mission for our industry to extend and share the science and technology, and also strong integration to the world. The 5th congress will also have Satellite Symposia this time to put together the science and industry more, where Evonik, Ecolab, Dupont, Ansell and Yem-Vit companies share their experiences and informations

Turkish poultry meat industry grew up around 50% in a very short period from 1.476 million tons in 2007 to 2.226 million tons in year 2018. This huge growth made an incredible increase in our export by 298% , from 151 thousand tons to 506 thousand tons in 2018, and reach 3.7% share in global poultry meat marketing.. So it is clear that Turkish Poultry Meat Industry makes valuable contribution to supply healthy, enough and stable foods for humans not just live in Turkey, but also live in other foreign countries.

As a representative of Turkish Poultry Industry which has become the world's 8th largest poultry producers, we together with organising and scientific committees have started to accomplish a higher quality congress as worthy of our country in April 2019. Many distinguished scientists as a keynote speaker will join us and talk on poultry meat production from farm to fork including all disciplines. The main theme of the 5th congress will cover new devolopments, environment friendly poultry meat production, challanges, safe and sustainable poultry meat production, meat processing, besides importance of poultry meat for human nutrition and well being..

We will be very happy to host all relevant people from all around the world in our traditional congress which reflects the science and technology side of Turkish Poultry Meat Industry. Additionally, we are pleased to announce that national and international scientists and researchers who join us to share their experiments, ideas and comments have been supported and encouraged. The location of the symposium, Antalya is one of Turkey's most popular holiday destinations. It's historical sites, exceptional beaches, restaurants and entertainment facilities would always make your stay enjoyable.

We really look forward to welcoming you to 5th International poultry Meat Congress which will be held in beautiful and historical Antalya, from 24 April to 28 April 2019 to obtain more and more benefits for humanity with science and industry hand in hand by discussing the innovations and changes of poultry meat production in most comprehensive way with the participation of leading scientists and experts.

With our best wishes to you all..

Dr. Sait KOCA
President of BESD-BİR

Prof. Dr. Necmettin CEYLAN
Chair of the Congress

KEYNOTE and ORAL PRESENTATIONS

OS[01] 5th International Poultry Meat Congress Opening Speech

Dr. Sait Koca
Chairman of Poultry Meat Producers and Breeders Association, BESD-BİR Ankara, Turkey

The fifth International Poultry Meat Congress that we held this year has become one of the most significant congresses on its topic. And again, this year our congress attracted intensive interest. Except spouses and children, there were more than 1000 participants as it was in every meeting before. On behalf of Turkish poultry sector, I would like to mention that we are delighted of hosting such a crowded and valuable group.

There are a limited number of hotels that have convenient halls for our congress. For this reason we have decided to get back to this exclusive place where we held the third of our congress.

Also in this year, unfortunately we lived hard times related to the last minute participation demands. However by the assistance of hotel management we were able to minimize this problem.

During the congress our entire team shall step up to the plate for the best and beforehand we request your tolerance for minor deficiencies that might happen.

This year we are hosting guests from 32 different countries. This is the most significant indication that reflects the international dimension of our congress.

Germany, USA, Australia, Austria, Azerbaijan, Belgium, Bosnia, Herzegovina, Brazil, Czech Republic, Denmark, Morocco, Finland, France, Holland, UK, Iran, Ireland, Spain, Italy, Canada, Turkish Republic of Northern Cyprus, Lebanon, Hungary, Malaysia, Egypt, Poland, Portuguese, Saudi Arabia, Tunisia, Turkey, Turkmenistan and Oman. Training is our most important priority as being “Turkish Poultry Meat Sector” and it has a great contribution to the development of our sector.

By this congress we altogether update our knowledge, learn about the latest developments, discuss the problems and generate solutions. The first day session of our congress will be in this hall. The second and third day sessions will be also be held in 3 different halls that will be formed in this same area. In total there will be 19 sessions. Besides 5 satellite symposiums are included in our congress program. Speakers who are experts in their field shall present 79 reports in 24 sessions in total. In addition there will be 57 poster presentations. Presents shall be awarded to the owners the posters ranking among the top three after evaluation and also to 3 people who have involved in the poster evaluation by casting lot.

Also 60 students from related faculties shall attend to our congress by scholarship.

All statements and their summaries that are presented in our congress are cited at to CAB Abstract Data Base.

CAB Abstracts is a data base that is developed by CABl institution based in UK and established in 1910 so as to spread free of charge information related to agriculture and life sciences to the beneficiaries. This is important for our Congress to be known and accepted worldwide. Therefore all of the statements included and presented in our Congress are accessible to all related parties and institutions all around the world.

The legal representative of poultry meat sector in Turkey is "Poultry Meat Producers and Breeders Association (BESD-BİR)".
Our Association was established in 1992 as "Breeders Poultry Association" and changed its name as "Poultry Meat Producers and Breeders Association (BESD-BİR)" in 1994 by the participation of poultry meat industrialists and become a wide based organization.

The scope of our Association can be summarized as; "to develop Turkish poultry meat sector, to create values that will contribute to the establishments, to represent the sector in the most correct way and to achieve communication between public administration and the industry".

BESD-BİR represents poultry meat sector of the highest order. It incorporates 23 member companies.
Hereby and once again I would like to thank to all of our member companies for their moral and material support to our association.

Our sector that is producing high quality poultry meat by applying modern and state-of-art technology has a vital role to close animal origin protein deficiency of our public. The most significant feature of the sector companies is to transfer all their income and also their capital to investment.

The leading poultry meat producer companies in our country that realizes almost 2/3 of the total production has established "**Healthy Chicken Information Platform**" in 2005 for the following reasons:

- ✓ Raise awareness of consumers, media and general public for the consumption of healthy poultry meat,
- ✓ Emphasize the importance of poultry meat for healthy nutrition and bring up to the agenda,
- ✓ Popularize international systems and high production standards during all production processes related to food safety at all companies,
- ✓ Provide accurate and reliable information to the public by the assistance and support of experts so as to correct wrong perceptions due to unscientific claims related to the sector.

The contribution of **"Healthy Chicken Information Platform"** to the sector is at ultimate level since the member companies of the platform give highest financial support to our activities.

I would like to thank to the member companies of **"Healthy Chicken Information Platform"** for their support in the name of the sector.

BESD-BİR is the founder member of International Poultry Council that is established in 2005.
IPC has 25 permanent members and 55 associate members.

World population is increasing. In 2008 world population was 6,6 billion people. However in 2018 world population exceed 7,6 billion. In 2050 it will become 9,77 billion and in 2100 it will be around 11,18 billion. The highest population increase is expected to be realized in the Africa continent. In Asia there will be a decline due to China.

Million	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
World	6.581	6.678	6.775	6.872	6.969	7.066	7.163	7.383	7.467	7.550	7.633

UN World Population Prospects: The 2018 Revision

Even if we consider an improvement in welfare the main agenda of the future shall be nutrition of increasing population and providing balanced diets.

Population of Turkey was 21 million in 1950 and it became 82 million in 2018. We are expecting the population to reach 104,8 million in 2050.

Million	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Turkey	71,5	72,6	73,7	74,7	75,6	76,7	77,7	78,7	79,8	80,8	82,0

TUİK

There are many global issues and while the current issues are waiting for a solution everyday new problems are occurring. The top 10 critical global issues according to the "2017 Global Shapers Survey" made by World Economic Forum are as follows:

1- Climate change - % 48,8
2- Large scale conflicts / wars - % 38.9
3- Income inequality - % 30.8
4- Poverty - % 29.2
5- Religious conflicts - % 23.9
6- Public accountability and transparency (corruption) - % 22.7
7- Food and water safety - % 18.2
8- Lack of education - % 15.9
9- Security - % 14.1
10- Inequality of economic opportunity and unemployment - % 12.1

According to the "World Food Unsafety Report" published by UN Food and Agriculture Organization (FAO) International Fund for Agricultural Development (IFAD) and World Food Program (WFP) (SOFI, 2017) the number of people affected from chronic food deprivation in the world by 2016 is 815 million. Due to the latest estimates for 2016, 155 million children under age 5 are suffering from growth retardation. It is another world fact that most of people who are deprived from chronic food safety are living in the regions that are affected from armed conflicts.

In 2018 the total amount of meat production was 335 million tons. 121.6 million tons of this production is poultry meat and it has a share of 36.3% in total production.

Poultry meat production in the world has reached its top level in 2015.

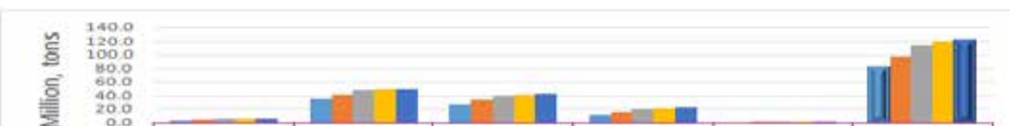
There are two main reasons why poultry meat production is growing faster than red meat production. First, it is environment friendly and second it has the most efficient feed conversion ratio. According to the FAO estimates, in 2025 total amount of meat production will reach to 358, 9 million tons and poultry meat will the most produced meat by 135.8 million tons, having a share of 37,8% in the total production. In 2018 the amount of poultry meat production was 121,6 million tons.

World Meat Production, million tons							
	2013	2014	2015	2016	2017	2018	2025
Cattle meat	68,0	68,0	67,6	69,7	70,9	72,3	77,5
Poultry meat	108,6	111,0	116,9	119,0	119,9	121,6	135,8
Pork meat	115,0	116,9	116,1	117,8	118,8	120,6	128,8
Sheep & goat meat	13,9	13,9	14,4	14,7	14,8	15,0	16,8
Total meat production	311,3	315,4	320,5	326,8	330,0	335,0	358,9

Food Outlook - November 2018
https://www.oecd-ilibrary.org/agriculture-and-food/data/oecd-agriculture-statistics_agr-data-en

In 2017, 40.8% of poultry meat production was achieved in American continent. Asian continent was at the second place and European continent was at the third place by 35.4% and 17.8%, respectively.

World Poultry Meat Production According to Mainland (Million tons)



	AFRICA	AMERICA	ASIA	EUROPE	OCEANIA	WORLD
2005	3.6	35.9	27.3	13.2	1.0	81.0
2010	4.6	41.8	34.5	16.1	1.1	98.1
2015	5.2	48.2	40.1	20.0	1.4	114.8
2016*	5.8	49.3	42.3	21.2	1.5	119.9
2017**	5.9	49.6	43.1	21.6	1.4	121.6

*) Approximately, **)Estimated — Food Outlook – November 2018

United States of America, China and **Brazil** are well ahead in world poultry meat production. These 3 countries realize 46.5 % of world production. **Turkey** has a 2.3% share in the world production.

Poultry meat production of some selected countries, except chicken leg, thousand tons

U.S.A.	18.208	18.510	18.938	19.361	19.546
BRAZIL	13.547	13.523	13.612	13.355	13.635
CHINA	13.561	12.448	11.600	11.700	12.650
E.U.	10.890	11.560	11.912	12.200	12.475
INDIA	4.115	4.427	4.640	4.855	5.100
RUSSIA	4.222	4.328	4.617	4.872	4.900
MEXICO	3.175	3.275	3.400	3.485	3.600
TAILAND	2.692	2.813	2.990	3.170	3.280
TURKEY	1.961	1.925	2.188	2.225	2.335
ARGENTINA	2.085	2.119	2.150	2.110	2.120
COLOMBIA	1.481	1.538	1.627	1.679	1.750
OTHERS	15.415	15.786	15.948	16.488	16.991
WORLD	91.352	92.252	93.622	95.500	98.382

USDA April 2019 LivestockandPoultry: World MarketsandTrade

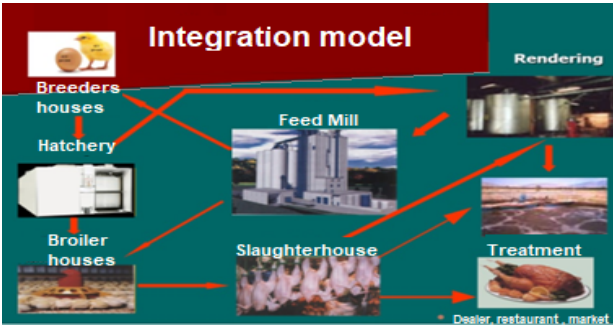
In 2018 poultry meat production in Turkey increased to 2.23 million tons. 2.16 million tons of this production is chicken meat and 0.7 million tons is turkey meat.

In Turkey, poultry meat production has increased between 2000 and 2018 by 3.4 folds and 1.5 folds between 2010 and 2018. 96.9% of the production is poultry meat and 3.1% is turkey meat. At least 95% of total poultry meat is chicken meat.

Turkey's Poultry Meat production (Tons)

Years	Chicken meat	Turkey meat	Total
2000	643.457	19.274	662.731
2005	936.697	42.709	979.406
2010	1.444.059	31.965	1.476.024
2011	1.613.309	36.331	1.649.641
2012	1.723.919	41.931	1.765.850
2013	1.758.363	39.627	1.797.990
2014	1.894.669	48.662	1.943.331
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

TUIK



Successful integration method used in poultry meat production has a significant role for the sector to reach its targets. Integrated establishments in the sector have breeder poultry houses, hatcheries, feed mills and slaughterhouses besides dynamic and strong marketing departments. All production chain is controllable by the integrations. Integrated companies have strong organizations, closely follow up technological developments and rapidly apply new technologies.

Also turkey meat sector realize production by integration model as chicken meat sector. The facilities that integrated companies own are more developed facilities that most of other countries and use state-of-art technologies.

Production in these integrated companies is realized according to international norms and "Food Safety" principals and continuously monitored by the official experts of Ministry of Agriculture and Forestry during all processes from breeder poultry houses to hatchery, from raw material producers to feed mills, from farms to slaughterhouse and from slaughterhouse to market shelves.

Regulation in Turkey is 100% compatible with the EU regulation.

Meat consumption per capita in the world is stable in the last years. Increase in production is only for the demand due to increased population.

Total meat consumption per capita in Turkey is below the world average. The most important factor for this below average consumption is no pork meat consumption as Turkey is a Muslim country.

Cattle meat and poultry meat consumption per capita in Turkey is above the world average, kg

	2014	**2015**	**2016**	**2017**	**2018**
Cattle meat	9,5	9,2	9,3	9,4	9,5
Poultry meat	15,5	15,8	15,9	15,9	15,9
Pork meat	16,3	15,7	15,8	15,7	15,8
Sheep and goat meat	1,9	2,0	2,0	2,0	2,0
Meat consumption per capita	**44,0**	**43,4**	**43,8**	**43,7**	**43,9**

Food Outlook – November 2018

Meat Consumption per capita in Turkey, kg

	2014	**2015**	**2016**	**2017**	**2018**
Cattle meat	11,4	13,1	13,3	12,5	12,9
Poultry meat	19,8	20,3	20,4	22,0	21,9
Pork meat	0	0	0	0	0
Sheep and goat meat	1,6	1,7	1,4	1,7	1,4
Meat consumption per capita	**32,8**	**35,1**	**35,2**	**36,2**	**36,2**

TUIK

As being the sector we aspired to close the meat deficiency due to pork meat and we spend all effort to realize our goal and proceed a lot.

All our effort is to close animal origin protein deficiency and provide healthy and balanced nutrition of our current and growing population, to allow our people to live a healthy life and to access animal origin protein with affordable prices.
We will keep going. Our objective is to increase poultry meat production and to enhance our country's meat consumption over the world average in a short time.

When we consider the quantities related to poultry meat consumption in the world we can clearly observe that many countries have a long way to go.

Countries in the top of the list of poultry meat consumption per capita, kg

	Country	Consumption, kg
1	Israel	58,2
2	USA	49,3
3	Malaysia	48,3
4	Australia	46,1
5	Saudi Arabia	42,3
6	Argentina	41,6
7	Brazil	40,7
8	Chile	40,1
9	South Africa	38,7
10	New Zealand	38,0

OECD FAO Agricultural Outlook 2018-2027

Poultry meat consumption per capita in Turkey has increased continuously and reached to 22 kg. Currently the rate of increase has slowed down and we can explain this situation by the uncertainty of the policies related to meat. Poultry meat has the biggest share in meat consumption. Turkey meat has also a great potential to increase the consumption

Poultry meat consumption per capita in Turkey (kg)

	Chicken meat	*Turkey meat*	*Total poultry meat*
2000	9,34	0,28	**9,62**
2005	12,79	0,58	**13,37**
2010	17,70	0,42	**18,12**
2011	18,48	0,46	**18,94**
2012	18,83	0,51	**19,33**
2013	18,18	0,43	**18,61**
2014	19,26	0,52	**19,78**
2015	19,66	0,60	**20,26**
2016	19,87	0,51	**20,39**
2017	21,44	0,56	**22,00**
2018	21,13	0,73	**21,86**

TUİK (Turkstat)

When we consider the world poultry exports, we can see that Brazil and USA are still the two biggest exporters in the world.

Related to China, it is considered that the exports will decrease due to the pork ASF disease problem that China is experiencing and the balance in global protein market is expected to change.

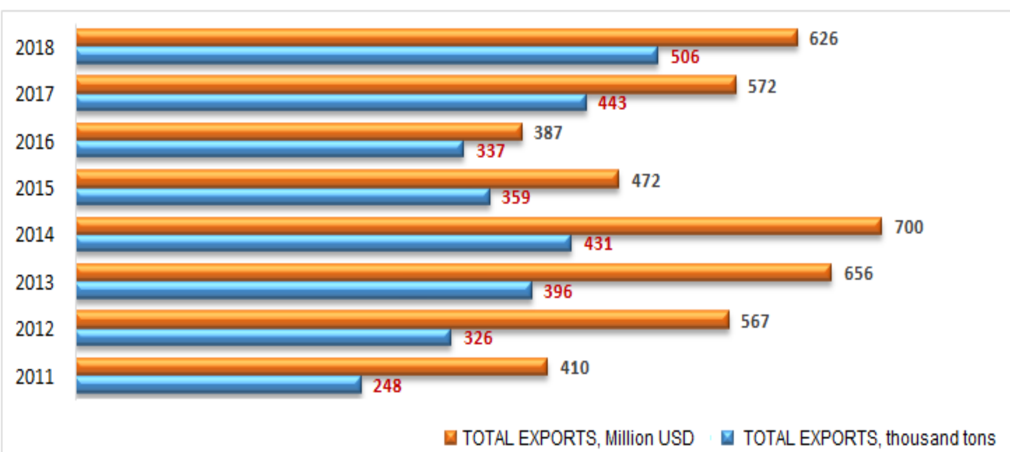
Exports of chicken meat exports of selected countries (except chicken leg), (thousand tons)

	2015	2016	2017	2018	2019 Estimate
BRAZIL	3.841	3.889	3.847	3.687	3.775
USA	2.932	3.086	3.140	3.244	3.272
EU-27	1.179	1.276	1.326	1.429	1.460
TAILAND	622	690	757	835	935
TURKEY	292	263	357	418	485
CHINA	401	386	436	447	425
UKRANIE	158	236	264	317	350
BELARUS	135	145	150	166	155
RUSSIA	71	104	124	128	135
ARGENTINA	187	158	178	124	130
CANADA	133	134	134	124	130
OTHERS	353	351	312	319	342
WORLD	**10.304**	**10.718**	**11.025**	**11.238**	**11.594**

USDA April 2019 Livestock and Poultry: World Markets and Trade

Poultry meat export of Turkey has increased considerably since 2008. We place great importance on exports as poultry sector. Turkey's export in 2018 has exceed 500 thousand tons including chicken leg. We have a 3.7% share in global trade.

Export of Poultry Meat in Turkey (including chicken leg)



UFT and Export Unions

We have exported to 81 countries in 2018. Iraq is the country where our exports are going. We work hard to enlarge current markets and to enter into new markets. EU is our target market and we worked intensively to get in this market since 2003. However legal permissions to export to this market are not completed somehow. When we compare Turkey with other countries that export to EU we know that we are not behind them but in fact we are in a more advanced position. Therefore we interpret the closed doors of EU to Turley as a political issue. And we do not have any hope anymore that EU will change this wrong political behavior.

The most important input of the sector is feed. Any problem related to the supply of feed raw material would cause a great differentiation from the target and at the same time an increase in cost. Then it would become hard for us to be competitive in exports. Turkish Grain Board is our greatest supporter and provides all and any kind of assistance and support within the scope of authority that it has according to the related laws. Unfortunately, mixed feed sector must import almost half of the raw material it needs. This means sector is open to price fluctuations due to any global price change.

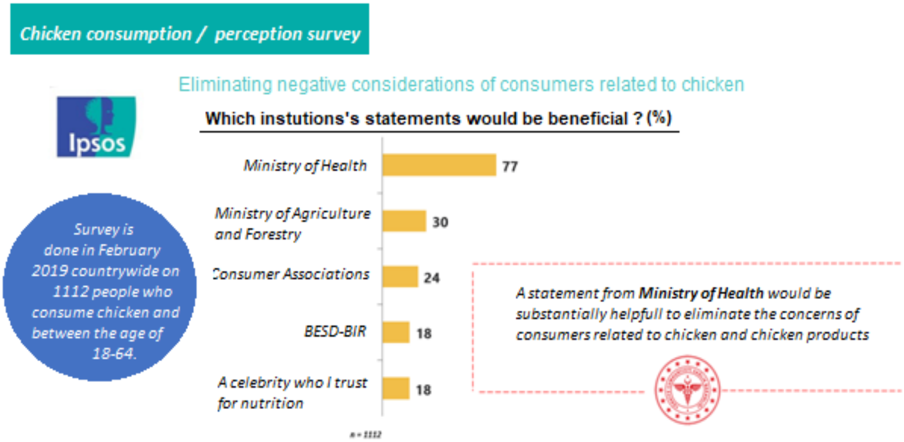
We have constant problems in raw material imports due to Biosecurity Law and related legal regulations.

The number of approved transgenic products in the world is 507 and 125 in EU. However approved transgenic product number in Turkey is only 36. Therefore we have to be very selective while importing and it increases our import costs.
It is inevitable that necessary amendments should have to be done in Biosecurity law and related regulations and they should be compatible with EU.

Statements that are published in written and visual media and which do not have any scientific background cause info pollution and seriously impair our sector. This issue is not limited only with our sector but also includes all sectors related to food production. It's not possible to understand why and how people can put such statements forward without knowing this sector that has a significant importance for our country.

Most of those who cause this info pollution are unscientific. They comment depending on fake scenarios. They never research or investigate anything that they are talking about and they do not consider what scientists say. We continue to do the right things as poultry sector and in the future, we will go on like this. In the course of time we believe that public shall decide about these people and the right party in other words we will win. The support of official authorities to our efforts is vital. We think that the statements of official authorities would be beneficial to eliminate the negative perception of the consumers.

According to results of the perception survey that we (BESD-BIR) want from IPSOS, first is the Ministry of Health and second is the Ministry of Agriculture and Forestry.



Under the guidance of BESD-BIR, a study related to “Decrease of antimicrobials used for treatments” has conducted in the beginning of 2016. In this study very successful results were obtained by the support of Ministry of Agriculture and Forestry and the study will continue in the following years.

There is a presentation in our congress related to the details of this study.

In 2018 BESD-BIR and Healthy Chicken Information Platform has organized cooking contest “Creative Recipes with Chicken” for the second time where many young, old, amateur or professional people who are interested in cooking all around from Turkey have attended.

The cook book “Creative Recipes with Chicken” is also published.

Turkish and English versions of the presentations at the 4th International Poultry Meat Congress are printed and submitted to all attendants. The book of this congress will also be submitted to you as soon as possible. We are also submitting the congress book and our other publications to the academicians of Departments of Agriculture, Veterinary, Food and Medical Faculties that are related to the sector.

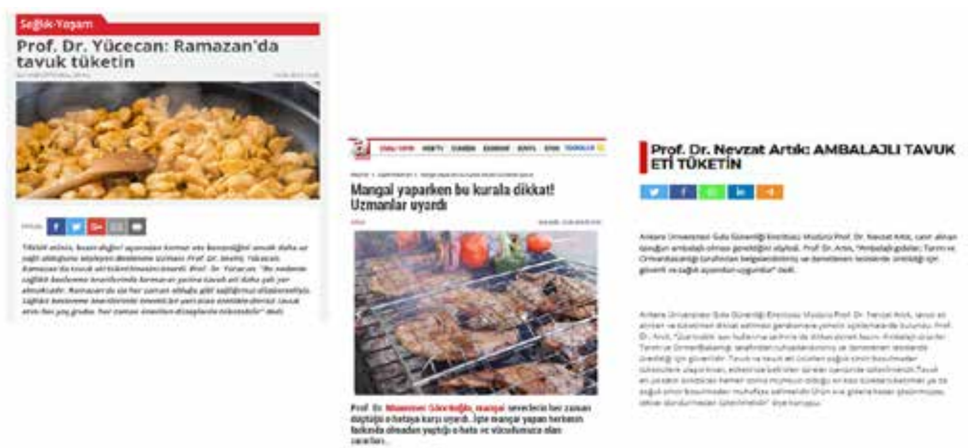
All our facilities are always open to all related people and institutions.

Lately, we host popular mothers and food blogger of social media, the managers and directors of Family Physicians Association and Federation at our facilities and we made a presentation related to our sector for Family Physicians Association and answered the questions of our visitors.

All through the year we will organize facility visits for different sections of the public.

In the last 2 years, we intend to correct the wrong information related to our sector by providing information under the title of “Lets speak the truths about poultry meat” at the reputable and large scale congresses where family physicians and nutritionists attend. We have reached approximately 11,000 physicians by attending to 9 congress in 2 years.

We organize regular informative interviews for public with the assistance of expert academicians.
We announce these interviews to the public with press releases.



In conclusion I say;

For healthy generations;

Our preeminent responsibility is to produce high quality and safe food…

In the name of Board of Directors, I would like to thank to, first Prof. Dr. Necmettin CEYLAN, Congress Chairman and members of Science Board, all who contributed to our Congress by their presentations and posters, our session leaders, Papyon Organization for this successful congress organization, all who support our Congress by purchasing stands, BESD-BIR General Secretary and staff, Mr. Ünal Akpınar the owner of our hosting hotel Starlight Convention Center &Sunrise Park Resort and all his staff and all who I cannot mention here but have put great effort for the realization of this organization.

I also would like to thank to you all for your interest and attendance to our Congress.
I wish it will be a successful congress.
Best regards and thank you…

IS[01] Role and Importance of Poultry Meat in Physical Growth and Mental Development of Children

Raşit Vural Yağcı
Ege University Medical Faculty, İzmir, Turkey

Our life starts with only two cells. That "2 cells" increase by 3 billion folds in 280 days. After we are born we grow up rapidly. In no period of our lifespan we do not grow by 3 folds just in a year. When we are at the age of one, our weight becomes 3 times more than the weight of us during the birth. In one year we become 25-27 cm taller. Our head grows by 10-12 cm. During adolescence, a rapid growth can also be observed but not in that scale. If we match this phenomenon with a machine we can say that higher the quality of the fuel, excellent is the performance of the machine. During this period development of the brain is extremely important. When we are born the weight of our brain is 330 grams. When we reach the age of 3, weight of our brain becomes 1 kg. At the figure below, you can see the development of the brain and everything happens in the first 1000 days or 3 years. After that there is only a section related to the development of social individual competencies during the pre-school period. Therefore if we can keep our brain rich of some fatty acids in the first 1000 days then there will be no performance deficiencies. Not only our brain develops. Also the microbiota develops. When we are in our mother's womb, there are only a few bacteria in the amniotic fluid. Infant starts to be acquainted with them. Bacteria also exist in the breast milk but infant should have to have sufficient number of bacteria when it is born so as to survive. Here, that sufficient number of bacteria increases rapidly by the birth process and support of breast milk. This is vital to protect the infant from acute diseases and chronic non-infectious diseases. This subject is critical to develop and protect a healthy immune system and

The first 1000 days is extremely important for an infant for physical growth, development of mental health (sanity) and a lifelong health. Infants who are fed and develop correctly during this period get ill less and even when they are sick they recover 10 times faster. They can live a life without any educational restrictions related to health issues. They can have minimum absenteeism at school. They gain minimum 1 year for that instance. Therefore any child who has lived these 1000 days in a good way and who is physically and mentally healthy will have a higher IQ level without any absenteeism at school and as a result could have a higher living standard (%22). Our aim is to increase the number of these people both in our world and in the world. When these people come together and form a family than the long term health and welfare shall also be determined.

6 advices for the first 1000 days for health

1. Healthy pregnancy
2. Normal delivery
3. Breast milk
4. Functional, fermented and natural products
5. Immunization
6. Life without antibiotic

If we can obey these 6 rules then it means that we spend these 1000 days correctly and provided that bifidobacterium dominate the microbiota. Healthy life program progress is supported by the development of appropriate intestine barrier function and immune response. The most precious present that a mother can give to her baby is her own bacteria and milk. Breast milk protects poor's child from malnutrition and rich's child from obesity. It is a human right to be fed with "breast milk". Currently "not to be fed with breast milk" is a global issue. Fed with optimal breast milk in the first age means 6 folds more living chance. We can prevent 1.4 millions of deceases under age of 5 by the optimal breast milk feeding in the first 2 years. The proposal of WHO (World Health Organization) is as follows;

- in the first 6 months only breast milk should be given, if not available then most appropriate milk can be given. Supplementary food should not be a priority.
- 30% of the calorie that the 6-9 months old infant gets should be from supplementary food.
- 50% of the calorie that the 9-12 months old infant gets should be from supplementary food and remaining should be from breast milk.
- main drink should be breast milk or most appropriate milk.

Sample menu for 6-12 months

Noon: meat (lamp/beef)/big as the palm of the infant, **chicken** – fish (big as the hand of the infant, vegetable (carrot – marrow – potato – tomato – artichoke – celery - jerusalem artichoke, portulaca / spinach), cereal (tarhana – noddle rice) soup. First vegetable cereal then meat (7th month).

Towards evening: fruit puree (pear, apple, banana, peach, apricot, plum…) then step by step yogurt (6-7 months). At the 7-8 months yogurt with egg yolk (benedict eggs) and fruit puree

At all other meals breast milk and/or formulated milk or cow/goat milk

Eggs for infant nutrition

Egg is the second best nutrient after breast milk. Its protein structure is very similar to the breast milk. It is very important for the development of brain, muscle and bones. Protects from anaemia. Start with hard-boiled egg at the 7-8th months. Glair is added to the menu at the 10-11th months. After age of one 1-2 eggs can be eaten.

Chicken meat for infant nutrition

It is the cheapest and most quality animal origin protein source together with egg. It is rich for vitamin B, phosphor, zinc and iron. Digestion is very easy. After 7 month it can be given to the infants. Cold chain is critical especially during summer months.

As a result;

- Mothers should be dissuaded from arbitrary delivery by caesarean section.
- Breast milk should be indispensable.
- In the absence of breast milk the most appropriate milk is breast milk or infant formula.
- Cow milk should not be given before age 1-2 unless it is obligatory. These milks do not contain protective bioactive ingredients in the breast milk and ingredients are not appropriate for infants.
- Homemade infant formulas made of water + rice flour + sugar are very inaccurate.
- Starting complementary feeding early and more dependency on that decreases and finishes breast milk and increase the risk of atopy – allergy and obeseness.
- An amount of 2-3 tea glass complementary feeding between 6-12 months is sufficient.
- Egg and chicken meat are the most efficient and economic protein sources.

IS02 Truth on Poultry Meat, Health and Cancer

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Incidence of cancer frequency in Turkey is similar to the frequency of cancer in the world and also in the developing countries. In Turkey the standardized cancer incidence rate according to age is 269,7 and 173.3 for men and women, respectively (per 100.000 people). In our country annually 98 thousand men and 63 thousand women contact with cancer and every year. 70.000 citizens die due to cancer. The most frequent types of cancer for men are liver cancer and prostate cancer and cancer related to tobacco still maintain its importance.

Meanwhile, the cost of early diagnosis, treatment and patient care is a real burden on the country's economies. Globally, 1,000 billion USD is spent for cancer while in Turkey the cost is approximately 3 billion USD. In 2030, it is estimated that the annual cost of cancer for Turkey will be 9 billion USD.

It is difficult to conclude net conclusions by considering diet-cancer relation because:

- There are thousands of chemical substances in a diet.
- There might be cancer initiator, stimulating and preventive factors in a diet
- Changing a parameter in a diet might affect everything and lead to difficulty to evaluate the other factors
- In most of the cancers there is long latent period. There may be differences related to diet habits during the diagnosis and beginning period of the cancer and the development period.

Obesity is a very significant problem in our country and 32 % of our citizens are obese. Mortality rate for the group with body mass index (BMI) is found out to be 52 % and 62% for men and women in all cancer types, respectively.

Food and cancer are among the issues that are mostly manipulated in the world and wrong and misleading information are very common in media. It is the most popular issue that fakers love.

The spectrum of the issue lies in a wide range from daily diet and nutrients to herbal products and alternative or after-treatment. Today 40 % of the cancer patients take alternative or after treatment in addition to chemotherapy without informing their oncologist. The economic scope and size of the alternative and aftercare products soared up and a new sector in the world raised.

Poultry Meat Consumption and Cancer

The incidence of cancer and poultry meat (chicken, turkey, etc.) has been evaluated in various epidemiological studies. Most of the studies can not indicate a relation between poultry meat consumption and cancer risk. Some of the epidemiological studies (case-control or cohort) show there is positive correlation between over consumption of poultry meat and increase in thyroid cancer.

The meta-analysis of various prospective cohort and case control studies could not show any correlation between over consumption of poultry meat and bladder cancer, breast cancer, colorectal cancer, uterine cancer, oesophageal cancer, lung cancer, ovarian cancer, pancreatic cancer, kidney cancer and lymphoma cancer. In 1294 patients with stabile high level prostate cancer, over poultry meat consumption progressed the disease (HR 2.26 and p = 0.03).

In 23 case-control and 11 cohort study, the analysis by considering the smoking factor showed that there is an inverse correlation between over poultry meat consumption and lung cancer development. A long term follow up study on 532 patients with pancreatic cancer and a control group of 1701 people showed that there is an inverse correlation between poultry meat consumption and pancreatic cancer development. In 27 studies including 12 cohort and 15 case control studies, a total of 23,703 patients with prostate cancer and 469,986 healthy people were evaluated by a long term follow up. The meta-analysis of these studies was based on 3 groups; Western countries, Asia and South America. The meta-analysis could not show any correlation between low or over consumption of poultry meat and prostate cancer (P 0.7). The general analysis could not show any correlation between over poultry meat consumption and prostate cancer risk at that 3 regions.

The famous Nurses' Health Study that 90 thousand pre-menopausal women participated is a very long term and cogent study where red meat as a protein source during the premenopausal period was replaced by poultry meat or poultry meat + legume. It was reported that breast cancer is less frequent at the people who consumed poultry meat or poultry meat + legume.

Red Meat and Cancer

It is a controversial topic why over red meat consumption increases cancer risk. Excess calories and fat consumption align with red meat consumption is guilty for obesity etiology. Another factor is heme iron that is the form of iron in muscles and it could be absorbed easily compared to the iron included in vegetables and cereals. Heme iron in red meat is prooxidant and increases oxidative stress (inflammation) and causes DNA damage resulting an increase of free hydroxyl radicals in the cell. It is reported that heme iron is specifically associated with metabolic syndrome, CAD, stroke, atherosclerosis, DM, Alzheimer, Parkinson and cancer etiology.

However, it should be strongly emphasized that the amount of red meat consumed in Turkey is far from being a factor causing cancer risk in healthy individuals. In Turkey red meat consumption per capita is only 12.4 kg and it is decreasing every other year. For the sake of comparison, red meat consumption per capita in U.S.A. is 52-53 kg. Therefore, it is totally wrong and misleading to propose the cancer risk for the agenda and divest our citizens of this valuable nutrition. We permit our patients diagnosed especially with colorectal cancer, breast cancer or uterine cancer to consume red meat only once in a week.

Why Poultry Meat Consumption Does Not Cause Cancer?

Heme iron that exists in red meat at an excess amount causes a carcinogen substance N-nitroso forms to develop in the cell. The content of heme iron in poultry meat is so low as compared to red meat. Epidemiological studies show that unsaturated fats decrease the cancer risk while saturated fats work in the opposite direction, i.e. increase cancer risk. Those who consume more poultry meat than red meat are individuals keeping a balanced diet and adopting a healthier lifestyle. 19 studies that research the correlation between meat (poultry and red meat) consumption and cancer risk are included in this meta-analysis. In all these 19 articles poultry meat consumption and cancer risk are reviewed. In this meta-analysis no correlation between more poultry meat consumption and colorectal cancer was stated but it was shown that more poultry meat consumption reduces rectal cancer risk. Nevertheless, an EPIC study (*J Natl Cancer Inst)* published in 2005 showed that more poultry meat consumption reduces colorectal cancer and rectum cancer risk.

What is A Healthy Diet?

- Red meat (should be cooked well and appropriately) and animal fat should be consumed less.
- Consumption of sausages, salami and cured/smoked meat and meat products should be avoided. _
- Minimum 1-2 portion of fresh or undercooked vegetable-fruit should be consumed.
- Fibrous food consumption should be maximized.
- Fish consumption should be increased (providing that fish are not from contaminated water ponds and polluted seashores)
- Salt and salty products consumption should be less.
- Sugar and sweet products should be consumed less.
- Wholegrain products, brown rice etc. should be preferred.
- Fried food consumption should be minimized as possible as it could be and if necessary, vegetable oil or olive oil should be preferred for frying. Butter should not be used as frying oil.
- Alcoholic beverages consumption should be avoided or minimized.
- More importantly poultry meat (chicken, turkey, etc.) should be consumed.

Food-Nutrition and Cancer

There are numerous epidemiologic studied showing red meat consumption increases cancer risk. Also there are studies that indicate consumption of more red meat increases the incidence of colon cancer, breast cancer and prostate cancer. However red meat is not a risk for our country since its consumption is low. Epidemiological studies show that there is a strong correlation between the amount of fat in the diet and the colon, breast and endometrium cancer.

Many epidemiological studies indicate that consumption of more poultry decreases the risk of many cancer types. Consumption of more poultry meat does not increase cancer risk. Although some of the studies show that more poultry meat consumption during adolescent period might increase the incidence of thyroid cancer it is only possible by a very high portion amount as 3-4 portions per day. More poultry meat consumption do not increase prostate cancer but if any it could progress the cancer.

Except minimizing alcoholic beverage consumption and protection from obesity there is no specific evidence that show diet could decrease cancer risk. In particular, there is no foodstuff that prevents or treats cancer when eaten or drunken. The most significant and efficient methods to prevent cancer are physical exercise, healthy diet, avoiding smoking, alcohol and other hazardous substances, preventing infections (i.e. HPV and HBV vaccines) and cancer scanning for early diagnosis.

IS03 Effects of Storage Conditions of Eggs on Embryonic Development and Survival

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Abstract

Prolonged egg storage before incubation is sometimes necessary and has been associated with longer incubation duration and lower hatchability, chicken quality at hatch and later life performance. These negative effects have been associated with an increase in the albumen pH and an increase in the percentage of apoptotic and necrotic blastodermal cells. Potential solutions to reduce or prevent the negative effects of prolonged storage on embryo survival and development can be divided into two categories: 1) increasing the number of blastodermal cells before or during storage; 2) decreasing the number of apoptotic and necrotic cells. This paper particularly discusses the first category. It can be concluded that pre-storage incubation, incubation during storage (SPIDES) and the extend of the warming profile prior to incubation are tools to stimulate the number of viable cells. However, each of these tools should be used with care, because overstimulating the embryo may lead to a too advanced morphological stage, which might decrease hatchability even more.

Introduction

Due to variable market demands for day-old chickens and limited hatchery capacity, storage duration of hatching eggs can vary between a couple of days and several weeks (1). Storage duration of hatching eggs longer than 7 days has been found to negatively affect incubation duration (2), hatchability (3,4), chicken quality at hatch (3, 5) and later life performance (6, 7), although not all these aspects were always clearly demonstrated. The severity of the negative effects of prolonged storage duration has been suggested to be breeder age dependent (1, 6, 7, 8). Yassin et al. (1) demonstrated a more negative effect of prolonged storage duration on hatchability in younger breeders (<32 weeks) than in older breeders (>36 weeks) during a storage period up to 13 days. However, Tona et al. (6) demonstrated a more negative effect of 7 days storage duration in a 45-weeks breeder flock than in a 35-weeks breeder flock on hatchability and body weight at slaughter age. Pokhrel et al. (4) demonstrated no effect of breeder age (32 vs 63 weeks of age) on hatchability after 14 days of storage, but, hatchability was more decreased after 21 and 28 days of storage in the older versus the younger flock. These ambiguous effects may indicate e that other aspects of storage, such as temperature, turning etc. might influence the results as well. This paper will focus on 1) embryonic mechanisms that are involved in the effects of storage duration and 2) potential (pre-storage) solutions that can reduce the negative effects of storage duration on hatchability, chicken quality and later life performance.

Egg Constituents, Embryo Morphology and Cellular Activity During Storage

During storage of hatching eggs, roughly two main things happen within the egg: 1) changes in the egg constituents and 2) changes in the embryo. Changes in the egg constituents include an increase of the albumen pH from approximately 7.6 at oviposition to approximately 9.0 after 4 days of storage (9). This increase in albumen pH is accompanied by an increase of CO_2 loss from the albumen and consequently liquefaction (10, 11). Additionally, the yolk pH is increasing as well during storage from approximately 6.0 to 6.3 till approximately 6.5 to 6.8 (11). The increase in albumen pH during storage is an important defense mechanism against penetrating bacteria. As a result of the increasing albumen pH, the vitelline membrane strength weakens, which might negatively influence the attachment of the embryo to the vitelline membrane and consequently may impair embryo development (12, 13).

Besides effects on egg constituents, prolonged storage is also influencing the embryo (blastoderm) morphology. At oviposition, embryo morphological stage varies between approximately EG-VIII and EG-XIII (3, 14, 16, 17), according to the morphological grading of Eyal-Giladi and Kochav (15). Reijrink et al. (3) and Fasenko et al. (14) did not found a change in morphological stage during 14-day storage, based on stereo-microscopically judgement. However, recent investigations from Pokhrel et al. (4), using 3D high-resolution episcopic microscopy demonstrated that the morphological stage of embryos during a 28-day storage period increased from EG-XI to EG-XIII on average in a young flock (32 weeks) and from EG-XII to EG-XIII on average in an old flock (63 weeks). Besides morphological stage, the diameter of the blastoderm seem to be also affected by storage duration. In 1968, Arora and Kosin (17) already demonstrated a reduction in blastoderm diameter following storage and recently, this was confirmed by Pokhrel et al. (4). Notably, the latter study (4) also demonstrated a larger blastoderm diameter in eggs from older breeders (63 weeks) than in eggs from younger breeders (32 weeks) and it appeared that the decrease in blastoderm diameter during a 28-days storage period was larger in the old flock than in the young flock.

Prolonged egg storage has been associated with an increase in blastodermal cell loss due to apoptosis and necrosis (4, 17, 18, 19, 20). The extent of this increase in apoptotic and necrotic cells has been associated with the storage temperature (4, 21), with a faster increase with higher storage temperatures. It can be speculated that the total number of viable cells passes a certain threshold during prolonged storage, because of apoptosis and/or necrosis, below which the embryo will die. Alternatively, it can be speculated that apoptosis and necrosis occurs in a certain area of the blastoderm (area pellucida or area opaca), which might result in incomplete or changed development of certain embryonic structures, resulting in an increase of early embryo mortality (4). The removal of apoptotic and necrotic cells requires energy and with an increase of apoptotic and/or necrotic cells due to prolonged storage, the embryo can run out of energy and die (22). It has been suggested that during storage, eggs have a kind of "physiological zero" temperature, under which no embryonic development takes place (23, 24). However, it can be questioned whether such a "physiological zero" exists, because Arora and Kosin (21) demonstrated already in 1967 that even at a storage temperature of 7.2^{o}C, mitosis, apoptosis and necrosis were increased with longer storage duration, which means that energy available in the embryo will be used for removal of the debris of apoptotic and necrotic cells (25). It can be suggested that an embryo during storage may experience a "physiological zero" based on its morphological development, but it appears hardly possible to have a "physiological zero" for cellular activity.

Based on the processes during storage described above, it can be suggested that embryo mortality during storage is related to one of the following aspects: 1) number of viable cells (passing a certain threshold) is too low or 2) a run out of energy as a result of apoptosis and/or necrosis. Consequently, solutions for reduction or prevention of the negative effects of prolonged storage should focus on reducing the sensitivity of the embryo to prolonged storage duration by increasing the number of blastodermal cells or on preventing apoptosis and necrosis. Because the first approach has obtained most attention during the last decade, we will focus in this abstract on this part.

Opportunities to Reduce Negative Effects of Prolonged Storage

To reduce the sensitivity of the embryo for prolonged storage duration, the number of blastodermal cells should be increased, prior to or during incubation. Opportunities to increase the number of blastodermal cells are:

1) Pre-storage incubation,
2) Incubation during storage or SPIDES and
3) Pre-incubation warming profile.

These three methods will be discussed shortly.

<u>Pre-storage incubation</u>

During pre-storage incubation, eggs are warmed before storage for a duration of 2 to 8 hours at a temperature of approximately 37.5-38.0°C. Thereafter, they are stored. Pre-storage incubation has been shown to result in an advance morphological stage of embryos (14, 26) and higher hatchability (14, 27, 28), although other studies did not show an effect on hatchability (26) or even found a negative effect (14). Reijrink (29) summarized all studies related to pre-storage incubation and concluded that indeed it can have a positive effect on embryonic development and hatchability, but only in the case the morphological stage after pre-storage incubation was between EG-X and EG-XIII (15). In case embryos were already in an advanced stage at oviposition, pre-storage incubation will further advance the morphological stage beyond EG-XIII and (strong) negative effects on hatchability were found (14). Risk factors to obtain embryos in a too advanced morphological stage are e.g. an older flock age, a high breeder house temperature, laying nests with litter instead of role-away nests (internal egg temperature remains higher for a longer period) or too long pre-storage incubation duration. Because of these influencing factors, pre-storage incubation should be used with care and in an optimal situation, embryonic morphological stage should be examined prior to any pre-storage incubation treatment to determine the necessity and/or the required duration of the pre-storage incubation treatment.

<u>Incubation during storage or SPIDES</u>

Temporary heating of eggs during storage, which mimic natural incubation of birds, might stimulate the morphological stage of embryo (20, 30, 31) and improve hatchability. Short periods of incubation during storage (SPIDES) has been used in several ways and durations. For example, Dymond et al. (20) used 4-hour warmings at 4 to 5 days intervals during prolonged storage and found indeed advanced embryonic morphological stages, more embryonic cells, a higher proportion of viable embryonic cells and higher hatchability? In longer stored eggs (> 8 days), SPIDES has been shown to increase hatchability (20, 27, 30, 31, 32). During SPIDES, a number of genes related to apoptosis are up or down-regulated (31), which suggests that SPIDES is related to the reduction of apoptosis and is stimulating the number of viable embryonic cells. Consequently, SPIDES can reduce the negative effects of prolonged storage on embryonic development and embryonic mortality, but the duration and frequency are important. The role of breeder age and the optimal pattern and duration of SPIDES is still unclear and is probably interdependent of each other.

Pre-incubation warming profile

Reijrink et al. (33) compared two linear warming profiles (4 or 24 hours) from storage temperature (approximately 18ºC) to incubation temperature (approximately 38ºC) in 4 and 13 or 14 days stored eggs. It was hypothesised that a slow (long) warming profile reduced the temperature shock (34) for embryos as it has been speculated that in long stored eggs this potential temperature shock might be more detrimental for apoptosis and/or necrosis and for embryonic development than in short stored eggs. Consequently, a slow warming profile might be more important for long stored eggs than for short stored eggs. Indeed, it was demonstrated that in short stored eggs, warming profile did not affect hatchability, but in long stored eggs, a slower warming profile resulted in a higher hatchability (Δ=3.3 to 3.6%) than a fast warming profile. In a consecutive experiment, Van Roovert-Reijrink et al. (35) showed that the embryonic morphological stage only changed when the temperature was higher than 28ºC. Based on this information, the pre-incubation warming profile was split up into two parts: from storage temperature (18ºC) to 28ºC and from 28ºC to incubation temperature (37.8ºC). It was demonstrated that particularly the duration of the second part was influencing early embryonic mortality. The slower the second part, the lower the embryonic mortality. This is probably due to a further increase in the number of viable cells and a reduction in the percentage of apoptotic cells by a slow warming profile (Broekmeulen et al., unpublished results).

Conclusions

Prolonged storage of eggs is sometimes necessary, but might negatively affect embryonic development, hatchability and later life performance. Solutions to reduce the negative effects of prolonged storage of eggs are to stimulate the number of viable cells or to reduce apoptosis and necrosis during storage. Pre-storage incubation, incubation during storage (SPIDES) and the extend of the warming profile are tools to stimulate the number of viable cells. However, each of these tools should be used with care, because overstimulating the embryo may lead to a too advanced morphological stage, which might decrease hatchability even more.

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O01 Effect of Post-Hatch Holding Time on Broiler Live Performance in Different Time Hatched Chicks

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Abstract

This study was conducted to determine of the effect of post-hatch holding time on subsequent broiler live performance of different time hatched chicks. Hatching eggs from commercial flock at 32 wk of age. Hatching time was divided into Early hatch of 471-474 h, Middle hatch of 487-489 h, and Late hatch of 497-501 h. Chicks were randomly divided to three groups, which chicks were placed to floor pens with feed and water after holding 30 h (28-32), 42 h (40-44), and 54 h (52-56) in the hatcher. BWL (Body Weight Loss) during holding period after emergence from the shell to placement time and yolk sac percentage of chicks were determined. BW was recorded at placement time (0), 7 and 35 d of age, and feed consumption and mortality were calculated for 0-7 d and 0-35 d period. Chicks in all groups were weighed at the same age relative to placement on feed and water to evaluate the effect of post-hatch holding. BWL was increased, but yolk sac percentage and BW were decreased due to increased holding period in hatcher ($P<0.05$). There was no significant difference in BW at 7 d, but BW of 35 d of chicks was decreased because of increased post-hatch holding time. Although the highest BW was observed from Middle hatched chicks at placement time, Early hatched chicks had significant higher BW at 35 d compared with Middle and Late hatched chicks ($P<0.05$). There was an interaction effect between hatch time and post-hatch holding time on 7 and 35 d BW ($P<0.05$). In other words, there was no significant difference among post-hatch time groups in Early hatched chicks ($P>0.05$), but Middle hatched and 54 h held chicks had significantly lower BW compared to 30 and 42 h held chicks in the hatcher ($P<0.05$). BW of 42 or 52 h held chicks was significantly lower than 30 h holding in Late hatched chicks ($P<0.05$). Consequently, post-hatch holding period didn't have the same effect on Early, Middle and Late hatched chicks in this study, and extended holding period didn't have a negative effect on broiler performance in Early hatched chicks unlike other hatching time groups.

Keywords: Hatching time, post-hatch holding, body weight, feed consumption, mortality

O[02] The Effects of Brooding Temperature on Growing Performance, Small Intestinal Morphology and Some Blood Parameters in Broilers

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Abstract

This study was carried out to determine the effects of brooding temperature on growing performance, small intestinal morphology and some blood parameters in broilers. A total of 480 one day old chicks (Ross 308) were used in the study. During the first week of growing period, control (32-33 ^{0}C) and lower (26-27 ^{0}C) brooding temperatures were applied. The chicks were ransdomly classified as two groups with 12 pens each treatment group (20 chicks/pen). During experiment, body weight, cumulative feed consumption, feed conversion rate and mortality were determined. At 14 days of age, blood and small intestinal (jejenum) sample tissues were collected. Blood cells (heterophils, lymphocytes, monocytes, basophils and eosinophils) were counted, villus length, villus width, surface area, crypt depth and *Lamina muskularis* thickness were measured on tissue samples. During growing period, birds in the control group were constantly heavier than the birds exposed to lower brooding temperatures, and the body weight at 42 days of age was found as 2897.1 g in the control group and 2615.1 g in lower brooding temperature group. Feed conversion rate was found as 1.74 and 1.83 in the control and lower brooding temperature groups, respectively. Morphological traits of villus were found to be higher in the control group when compared to the other group. Villus length was found as 498.8 µm in the control group and 375.2 µm in the lower brooding temperature group. The ratio between heterophils and lymphocytes was higher in the lower brooding temperature group than the control group (1.63% vs. 1.44%). As a conclusion, lower brooding temperatures applied during the first week of growing period increased stress level of birds, depressed intestinal villus development, finally caused a deterioration of growing performance of broilers.

Key words: brooding temperature, villus, stress, heterophils/lymphocytes ratio, broiler

IS[04] Effects of Processing and Enzymes on Cell Wall Degradation and Nitrogen Utilisation in Poultry

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Summary

During feed processing, the physicochemical characteristics of fibre fractions from the feed can be modified, thereby affecting the degradation of dietary fibre and its possible interference with digestive processes in the bird. Commonly used mechanical and thermal feed processing methods, such as milling and pelleting, reduce particle size and increase solubility of non-starch polysaccharides and thereby may improve degradability of fibre fractions by 0 to 16 % units. The effects are, however, limited to rather easily solubilisable NSP as found in barley and wheat, whereas more recalcitrant fibre fractions, such as found in maize, in several oilseeds, and in their by-products, are hardly affected.

Apart from the effects on fibre degradability, modifications of the physicochemical properties of fibres by processing may also affect digesta properties and transit through the GIT. For example, thermal processes generally increase viscosity of diets rich in soluble β-glucans, arabinoxylans, and pectins, corresponding to increased small-intestinal digesta viscosity in the bird. Furthermore, altered rheological properties of digesta resulting from processing, may reduce gizzard activity and retention time but likely also influence digesta transit behaviour beyond the gizzard.

Introduction

Although the importance of dietary fibres to contribute to the nutrient supply in chickens is rather limited, fibres may interfere with the digestion of other nutrients through (1) encapsulation of nutrients in the cell wall matrix and (2) their effects on physicochemical properties of the digesta, thereby influencing nutrient accessibility, bulking properties, microbial activity, gut physiology and function, endogenous secretions, and transit of digesta through the gastrointestinal tract (GIT)[1-7]. For example, various soluble fibre sources have been found to reduce digestion of protein, starch, and fat in the upper GIT[8-10], whereas insoluble fibre sources as oat hulls, may improve nutrient digestibility[11, 12]. The adverse effects of soluble fibres are mainly explained by their viscous nature, and fibre-degrading enzymes (as β- glucanases and xylanases) are generally added to poultry diets to reduce these effects. The beneficial effects of insoluble fibres likely result from improved activity of the muscular gizzard and are generally ascribed to the physical structure of the fibrous ingredients. Hence, fibres or coarse particles are often added to the feed to improve gastrointestinal functioning and nutrient use[reviewed by 13, 14].

During feed processing, the physicochemical characteristics of fibre fractions, such as particle size, solubility, and hydration properties, can be modified. Therefore, the degradation of dietary fibre and its possible interference with digestive processes in the animal, may be affected by feed processing technologies. Modifications in chemical structure and physicochemical properties of fibres caused by processing are, however, poorly understood and strongly depend on processing conditions and the type of fibres [15, 16].

Effects of Processing on Physicochemical Properties and Degradability of Fibres

The degradation of non-starch polysaccharides (NSP), the major constituents of dietary fibre, in the GIT largely depends on the original cell wall matrix in which the polysaccharides are embedded[16]. Generally, polysaccharides that are more easily solubilised from the cell wall will be more easily degraded; they are not firmly anchored in the cell wall matrix and are therefore better accessible by microbial enzymes. Contrary, NSP that are firmly bound to lignin are usually more recalcitrant[17]. In chickens, ceca access is restricted to fluids and small particles (<0.2mm) [18-21] and only fibres present in the soluble or fine digesta fractions, reach this major site of fermentation. It follows that digestibility coefficients of NSP observed in chickens are generally related to the solubility of the NSP fractions in the diet[12]. Commonly used mechanical and hydrothermal feed processing methods may reduce particle size and increase solubility of NSP and thereby improve degrabilty of fibre fractions, as reviewed by [16]. It should be noted that evaluation of effects of processing on the degradability of fibrous fractions should preferably focus on the total NSP or total dietary fibre fraction instead of the less specific crude fibre (FF), neutral detergent fibre (NDF), and acid detergent fibre (ADF) fractions. For processing will alter the physicochemical properties of samples and consequently fibre components that are recovered in CF, NDF, or ADF fractions, will differ before and after processing.

Dry thermal processes, such as infrared radiation, have minor effects on physicochemical properties of the fibre fraction, and, thus, marginally affect fibre degradability in the bird[16]. Milling and hydrothermal processes such as expander processing and extrusion cooking operate high shear forces. These forces can effectively disrupt the cell wall matrix and, consequently, improvements in fibre degradability in broilers have been reported to range between 0 and 16% units, with an average of 7% units[16]. The solubilisation of NSP from the cell wall matrix depends on the covalent and non-covalent cross links between the various polysaccharides and other cell wall components. This explains, why the effects of processing on fibres may differ among sources. For example, β-glucans, arabinoxylans, and pectins from primary cell walls are easily solubilized by milling and mild hydrothermal processes. Contrary, solubilisation of polysaccharides from secondary cell walls is often hampered by intermolecular ionic bridges, ester linkages, and hydrogen bonds with cellulose and lignin, and thus require more severe processing conditions. Hydrothermal treatments under neutral and alkaline conditions promote β-elimination of mainly highly esterified pectins, whereas acidic conditions favor hydrolysis of polymeric pectins with a low degree of esterification [16, 22, 23].

To conclude, common feed processing technologies only affect degradability of rather easily solubilisable NSP, such as cereal β-glucans found in barley and wheat. The effects on rather recalcitrant fibre fractions, such as found in maize, in several oilseeds, and in their by-products, are, however, limited [24-27]. Hydrothermal pre-treatments using acid or alkali catalysts are established methods to degrade lignocellulosic material [28, 29] and can potentially be used to modify such recalcitrant fibre fractions. For animal feed applications, however, processing temperatures and concentrations of acid and alkali to be used, are limited due to potential protein damage and the high residual concentrations of chemicals. Future research should aim at targeted degradation of recalcitrant fibre structures, while minimizing effects on easily solubilisable NSP and other nutrients. Recent research in maize dried distillers grain with soluble and rapeseed meal indicated that ester-linkages or H-bonds are responsible for the recalcitrance of the fibre fractions in these ingredients, presumably due to their involvement in anchorage of NSP in the rigid cellulose-lignin matrix [25, 27, 30]. Hence, technologies that degrade such linkages, as alkali treatments or esterases could be of interest for future research.

Effects of Processing on Digesta Properties and Transit Behavior

Apart from the effects on NSP degradability, modifications of the physicochemical properties of fibres by processing, may also affect digesta properties and transit through the GIT, thereby potentially affecting nutrient degradation. The solubilisation and partial degradation of fibres during processing can affect their viscous properties, thereby altering their effects on the digesta matrix. Particle size reduction by milling generally reduces extract viscosity, due to (partial) degradation of polysaccharides, thereby reducing junction zones between the polysaccharides and the formation of viscous networks. During thermal processes, however, NSP are often solubilised without actual degradation of the polysaccharides. Hence, thermal processing often increases viscous nature of NSP, particularly soluble β-glucans and arabinoxylans, and pectins[16], corresponding to increased small-intestinal digesta viscosity[31-36]. In these cases, the addition of cell-wall degrading enzymes can successfully counteract such increases in digesta viscosity[16].

As often emphasized, fibres play an important role in the regulation of digesta transit in the upper GIT [11, 13, 14], and reduced particle size, resulting from mechanical and thermal processes, may indeed reduce gizzard activity and retention time[34]. However, fibres seem also significant for the regulation of digesta transit in other GIT segments. The meshwork of ridges and villi at the opening of the ceca, prevents coarse particles from entering and backflow of digesta from the cloaca into the ceca, seems to be restricted to soluble fraction. Thus, rheological properties of the digesta likely also influence hindgut reflux and ceca filling[37, 38]. It seems evident that processing may, therefore, also affect gastrointestinal transit of digesta beyond the gizzard. To our knowledge, studies on the effects of processing technologies on GIT retention time of digesta are scare and effects on cecal retention times have not been reported. Nevertheless, the fraction of NSP that reached the ceca when fed to broiler chickens, was greater for finely ground (geometric mean diameter: 220 μm) than for coarsely ground rapeseed meal (geometric mean diameter: 818μm)[24], indicating that indeed processing .

Conclusion

Commonly used mechanical and thermal feed processing methods, such as milling and pelleting, reduce particle size and increase solubility of non-starch polysaccharides and thereby may improve degradability of fibre fractions by 0 to 16 % units. The effects are, however, limited to rather easily solubilisable NSP as found in barley and wheat, whereas more recalcitrant fibre fractions, such as found in maize, in several oilseeds, and in their by-products, are hardly affected.

Apart from the effects on fibre degradability, modifications of the physicochemical properties of fibres by processing may also affect digesta properties and transit through the GIT. For example, thermal processes generally increase viscosity of diets rich in soluble β-glucans, arabinoxylans, and pectins, corresponding to increased small-intestinal digesta viscosity in the bird. Furthermore, altered rheological properties of digesta resulting from processing, may reduce gizzard activity and retention time but likely also influence digesta transit behavior beyond the gizzard.

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O[03] Combination of NSP Enzyme and Phytase Super Dosing Improves Growth Indices and Carcass Traits in Ross 708 Broilers

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A 42 day broiler growth trial was conducted to evaluate the impact on live performance, carcass quality and woody breast severity in Ross male 708 broilers when fed a combination of NSP enzyme (Hostazym® X) 1500EPU/kg and a single dose of phytase (OptiPhos®) 500 FTU/kg or a super dose of 3000FTU/kg, this was compared to a standard dose of phytase (OptiPhos®) 500FTU/kg Positive Control (PC) and a Negative Control (NC) the NC feed was reduced in dietary energy by -100Kcal/kg broiler AME.

A completely randomized design with four treatments based on Corn/Soya/DDGS using 95% Ross genetic nutritional recommendations for the 708 broiler. These diets were subsequently fed to 828 Ross 708 Males broiler chickens. The following diets 1). Positive control (PC), 2: Negative control (NC-100Kcal/kg AME) were fed to 8x replicates x 23 birds/pen, the test treatments 3). (NC)+ Hostazym® X (1500EPU/kg) & OptiPhos (500FTU/kg) & 4). (NC)+Hostazym® X (1500EPU/kg) + OptiPhos® (3000FTU/kg) were fed to 10 x replicates x23 birds/pen. Carcass quality was assessed at the end of the trial.

Birds fed the NC diet were the worst performing compared to all other test diets ($p<0.05$) for body weight, and FCR, birds fed Hostazym® X enzyme and 500FTU/kg OptiPhos® phytase demonstrated the least incidence of woody breast ($p<0.05$) compared to all other treatments tested.

Overall the inclusion of Hostazym® X and OptiPhos® as a combination of phytase and NSP enzyme positively influenced all growth performance parameters and carcass quality traits compared to the PC and NC diets.

Keywords: Phytase, NSP enzyme, carcass quality, performance

O[04] Hydrolysis Characteristics of Purified Peptidoglycan from Chicken Gut Bacterial Isolates by a Fungal Muramidase

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Introduction

The cell wall of all bacterial species present in the gastrointestinal tract comprises of the structural polymer peptidoglycan. *In vivo* supplementation of a novel fungal muramidase enzyme, capable of hydrolysing peptidoglycan (PGN) remnants, has been reported to significantly improve performance parameters in broiler chickens (1, 2). However, the preference of this novel muramidase for PGN remnants from the different gut relevant bacteria is still not fully understood.

Material and Methods

Peptidoglycan from five relevant chicken gut bacteria (*Enterococus gallinarum*, *Lactobacillus aviaries subsp araffinosus*, *Lactobacillus kitasatonis*, *Bifidobacterium gallinarum*, *Lactobacillus gallinarum)* was extracted and partially purified. The novel muramidase was tested in combination with these five PGN extracts to study their hydrolysis characteristics using reducing sugars end assay, mass spectrometry and a PGN detection assay (silk larvae plasma assay).

Results and Discussion

The three applied methods confirmed that the fungal muramidase was capable of hydrolysing peptidoglycan extracts from all five bacteria. The number of reducing sugars significantly increased for all five peptidoglycan extracts upon hydrolysis. The apparent highest response to muramidase supplementation was observed for *L. aviaries*, whereas the other four peptidoglycans showed similar response. Soluble degradation products of the muramidase-assisted hydrolysis reaction were measured by mass spectroscopy. 21 out of the 93 identified peptidoglycan degradation products were significantly more abundant across the five substrates following hydrolysis. Muramidase-hydrolysed peptidoglycan extract from all five bacteria activated the silk larvae plasma assay significantly less compared to the untreated substrate, indicating an effective PGN hydrolysis and loss of structural conformation.

Conclusion

Three different *in vitro* assays showed that a novel muramidase is capable of hydrolysing PGN extract from five different chicken gut relevant bacteria.

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O[05] Isoquinoline Alkaloids in Poultry Production – a Review

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Introduction

Nowadays, phytogenic feed additives are common practice in many countries to strengthen gut health in food producing animals. Inflammation management may lead to an improved gut health and therefore contributes to a sustainable, economical and efficient poultry production. Isoquinoline alkaloids (IQs) are known for their anti-inflammatory properties (1). A standardized blend of IQs is being widely applied across poultry production systems worldwide (Sangrovit® product line). IQs were tested in a series of *in vitro* and *in vivo* trials to evaluate their anti-inflammatory effects as well as their effect on broiler´s performance.

Material and Methods

The presentation will summarize research on IQs conducted in *in vitro* and *in vivo* trials. In addition, a meta-analysis will be presented. A total of 22 university trials were conducted. The trials were carried out in 18 different countries, using more than 30,000 broilers. Daily weight gain and feed conversion ratio were evaluated, comparing a negative control to birds fed IQs (2).

Results and Discussion

The transcription factor NF-κB is considered to play a crucial role in the inflammatory response. Once NF-κB becomes activated, the transcription of pro-inflammatory cytokines is triggered (3). Consequently, acute phase proteins, tight junction proteins and cortisol levels are affected. IQs can inhibit NF-κB (1). Consequently, effects on cytokines were seen, (4), if IQs were supplemented. As a consequence, a beneficial shift in the microbiota could be achieved (5), less inflammatory cells were activated (6), and performance of birds was improved during an infection with *Clostridium perfringens* (7). The results *in vivo* and *in vitro* underline the anti-inflammatory effect of IQs. By contributing to gut health, IQs might affect broiler´s performance in a positive way. The results of the meta-analysis indicated that body weight gain was improved on average by 4.0% compared to the negative control group, if IQs were supplemented. Feed conversion was improved on average by 3.1%, if IQs were fed to broiler chickens.

Conclusion

IQs have a defined mode of action and display beneficial effects on broiler´s performance. Therefore, IQs provide a beneficial and sustainable solution to the poultry industry.

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IS[05] Mycoplasma Synoviae from the Primary Breeder's Perspective

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Summary

The impact of *Mycoplasma synoviae* on broiler breeders is mainly through losses in the broiler progeny as a result of chronic disease, reduced performance and increased condemnation rate at the processing plant. Options for monitoring and control in broiler breeders are discussed.

Avian mycoplasmosis is a frequently reported infectious disease in poultry. The two species of Mycoplasma implicated in chickens are *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS). These infections may cause chronic disease of the respiratory tract, locomotor system and may result in significant economic losses due to decreased bird performance, increased medication costs and downgrading of carcasses at the processing plant.

Infectious synovitis of chickens was first reported in the United States by Wills and others in 1954. MS has worldwide distribution and for a long time it was considered the second most important mycoplasma affecting chickens (Stipkovits & Kempf, 1996). This view has shifted slightly over the years as MS has become more widespread causing significant issues in many countries (Is Mycoplasma synoviae outrunning Mycoplasma gallisepticum? A viewpoint from the Netherlands, Landman, 2014). The incidence of seropositivity in commercial egg layers may range from 70 to 90% (Landman, 2014).

MS can cause a wide range of clinical problems such as egg yolk peritonitis, egg production losses including smaller eggs, cracks and downgrades, Eggshell Apex Abnormality (EAA) in commercial layers and amyloid arthropathy in brown layers (Landman et al., 2001). The hatchability can also be affected in layer and broiler breeder parents (Stipkovits and Kempf 1996).

Broiler breeders are rarely affected directly though and MS mostly remains a silent infection. The economic impact affecting the meat chicken industry is the result of vertical transmission of MS to the broiler progeny and a wide range of different manifestations of the infection, which may be strain related: infectious synovitis (lameness and runts), respiratory disease (including airsacculitis, also in day-olds), increased mortality as a result of chronic respiratory disease (CRD), decreased weight gain, poorer feed conversion, increased medication costs and higher condemnation rates. Stress is an important factor in precipitating disease (Bradbury). Furthermore there is evidence that synergism with other viral respiratory and bacterial pathogens exists e.g. with IBV, NDV (even respiratory viral vaccines) and avian pathogenic E. coli (APEC). The incubation period is 6 and 2-21 days after vertical and horizontal transmission respectively.

Mycoplasma are fragile organisms and usually do not persist in the environment for longer periods of time because they rely on a host organism to survive. Most detergents and disinfectants kill mycoplasma successfully. It is not stable at pH 6.9 or lower and sensitive to temperatures above 39 °C. Carryover of infection from an infected flock on a single age, all-in all-out site after appropriate and thorough cleaning and disinfection should not occur.

The main natural host species are the chicken and the turkey. MS has also been isolated from a number of other avian species including the pheasant, quail, partridge, guinea fowl, geese and ducks.

MS can be transmitted vertically or horizontally. Primary breeders and higher generation flocks (Pedigree, GGP and GP) must remain free from MS. It is desirable that broiler breeders (PS) are kept free from MS, too but this might not be achievable for some farms or regions with high poultry density. Horizontal transmission is usually through direct or indirect contact but airborne spread through aerosol and dust are thought to be one of the most important mechanisms e.g. when slurry, litter is spread, scattered or during transportation of live birds, mainly spent fowl (laying hens or other infected poultry). Indirect contact with fomites, clothing and hair is another important aspect of transmission e.g. contact with infected poultry without any downtime prior to visiting breeders.

Clinical signs and positive serology can be suggestive of MS. In broiler breeders however symptoms are rarely observed. That is why regular MS monitoring is essential in breeders. Seroconversion can take 7-10 days post infection (p.i.) depending on the test kit used. Confirmation should be by polymerase chain reaction (PCR). Culture can also be used but that requires suitable sampling kits, transport media and specialized labs.

Flock Monitoring

Serology

Correct sample size, statistically valid sampling methods, good serum quality and reliable serological tests are essential for an effective monitoring programme. ELISA tests are widely available, convenient, reproducible, high throughput and cost effective. Some test kits can detect antibodies as early as 7 days p.i. while the level of false positive results can be kept reasonably low (<0.5%). The rapid slide agglutination test (RSA) can also be used. It detects circulating antibodies 10 days p.i. and the rate of false positives is around 1%. Never use frozen sera for RSA. Heat inactivation and dilution of sera may help troubleshooting false positives. Another approach is the collection of dry throat swabs during blood sampling. These "storage" swabs may only be tested by PCR in case the serology methods don't deliver clear results. Re-bleeding of the flock a few days later may also be considered. Systemic bacterial infections (e.g. E. coli peritonitis), the recent use of viral or bacterial inactivated vaccines could trigger false positive reactions as well. It is worth postponing blood testing for a couple of weeks or more in such cases. Another word of caution: avoid serological testing of day-old or young chicks because false positive results are very common.

PCR

There are many different commercial and non-commercial endpoint and real-time or qPCR assays available. PCR technology may be used as part of the monitoring programme. Sterile cotton or synthetic fibre buds on plastic or aluminium sticks (shafts) can be used to collect dry swab samples from the palatal cleft (choana) or the trachea. Wooden swabs are not appropriate for this purpose. Avoid contact with feed, litter or other contaminants. Insert the swab carefully so as not to damage the cleft and instigate bleeding. Swabs contaminated with blood should not be tested. A slight pink discoloration however is acceptable. The cost of testing can be reduced by pooling the samples e.g. if 16 swabs were collected from a house, every 4 could be assigned to 1 pool i.e. only 4 of these pools would be tested not the 16 individual swabs.

Minimum Recommendation on Monitoring Broiler Breeder Parents. Testing sera from day-old or young chicks is not reliable. Testing could start at 3-4 weeks of age and then repeated a couple of times in rear. Birds should be tested before the start of lay and then at least every 8 to 12 weeks depending on infection pressure. 30 or 60 blood samples per airspace should be taken to detect 10 or 5% prevalence respectively with 95% confidence. As discussed above, PCR assays could be used to complement serology or at least as a confirmatory test.

Options for Control

Mitigating Conceptual Biosecurity Risks. Distance is the main factor to prevent airborne spread. Hills and woodland, if present may act as natural barriers. Choosing an isolated location for a new breeder farm far away (at least 2 km) from any layer or broiler units will be of advantage. Single age farming with all-in and all-out management is another important aspect of maintaining freedom from MS. Bird buildings should be wild bird proofed.

Mitigating Procedural Biosecurity Risks. Only farm staff, other essential personnel and previously authorized visitors should enter the site. It must be ensured that visitors, including other essential personnel, have not visited any other poultry units for a set minimum period of time e.g. 48 hours. Ideally staff should have no contact with other avian species. Entry procedures including full body shower and changing into clean clothing provided by the farm should be considered. Spiking (introduction of young males into mid-lay breeder flock) should be avoided. If it is unavoidable it has to be ensured that males are from an MS negative flock. The crates and bird move vehicles for rear-to-lay or male transfers must be properly cleansed and disinfected. The routing of the bird transport vehicle must be such that it avoids layer and broiler units. The bird move crew must observe relevant biosecurity rules including pre-visit movement restrictions and entry protocols. Irregular activities should be managed so as to minimize the risk of contamination e.g. safe disposal of double yolk and reject eggs and cull males or non-layer hens just to name a few. Feed spillages must be immediately removed to not attract wild birds.

Treatment with Antibiotics

Note: Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, AMR is an increasingly serious threat to global public health that requires action across all government sectors and society (WHO). Therefore antimicrobials should be used responsibly. Suitable antibiotics may reduce clinical signs, performance losses and vertical transmission but relapses occur frequently. Antibiotics are usually given for a few days every 4 weeks. Minimum inhibitory concentration (MIC) testing may be required to establish which antimicrobial product is the most effective one. For MIC testing MS needs to be cultured. The efficacy of treatment should be monitored by PCR testing and if the flock becomes negative after 1 or 2 treatments it can be ceased. β-lactams (e.g. penicillin) and sulphonamides are not affective against Mycoplasma. Suitable antibiotics are macrolides, pleuromutilins, lincosamides, fluoroquinolones and tetracyclins. Please note that antibiotics in flocks where live vaccines are used for control will kill the vaccine and reduce the efficacy of vaccination.

Vaccination

When MS cannot be controlled through other efforts such as biosecurity and it is economically not feasible to eradicate positive flocks, vaccination should be considered.

Bacterins are killed vaccines that contain the whole pathogen as antigen and are usually oil adjuvated and have to be injected to each bird before the onset of lay typically twice. Bacterins may give moderate protection by preventing clinical signs but they do not prevent infection or shedding. A humoral immune reaction is elicited but that is not proportional to protection. Maternal antibodies do not protect progeny chicks from becoming infected. The downside also is that serological monitoring is not possible in vaccinated flocks.

Live, attenuated vaccines on the other hand reduce air sac lesions, ovary lesions, and a drop in egg production. Chickens have to be vaccinated before field challenge and should be used in flocks older than 5-6 weeks. It is recommended that MS status is confirmed by PCR before vaccination. The induced protection is efficient and long lasting. Vaccine strains colonize the upper airways triggering continuous antigen stimuli and mucosal (local) immunity. The use of live attenuated vaccines may help reducing antibiotic use. This type of vaccine does not spread vertically but it does horizontally. The onset of immunity is about 4 weeks and the duration usually longer than 40 weeks. No antibiotics should be used 4 weeks after vaccination.

Examples for live vaccines registered in the EU are MS-H Vaccine, a frozen product that requires being stored on dry ice or in -80 °C freezer (MS-H strain, Bioproperties Pty Ltd) and Nobilis MS Live freeze-dried vaccine (MS1 strain based on WVU 1853 field isolate, MSD Animal Health).

Differentiating Infected from Live MS Vaccinated Animals (DIVA testing). Serological tests cannot be used for DIVA testing but a number of different molecular methods are now available (Shahid et al., 2014, Kreizinger et al., 2015, Dijkman et al., 2017, Kreizinger et al., 2017).

O[06] Multiplex Quantitative Real-Time PCR Developing Rapid Diagnostic Kit for The Detection of Discrimination and Marek's Disease Vaccine Used in Turkey

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Abstract

Marek disease is a lymphoproliferative and highly contagious disease characterized by domestic birds, demyelination of peripheral nerves and mononuclear infiltrations. Marek is found in all poultry farming countries. Marek's disease virus is the first successful vaccine developed oncogenic virus in the late 1960s. Vaccines currently used are vaccines derived from live three viral strains: HVT FC126 strain, GaHV-3 SB-1 strain and GaHV-2 CVI988 / Rispens strain. The CVI988 / Rispens vaccine is a naturally attenuated strain of MHV-1 and the CVI988 / Rispens vaccine cannot be easily distinguished from virulent strains of Marek's disease. The vaccine cannot prevent infection with certain virulent viruses, as MHV vaccine strains provide effective infection and lifelong immunization and effective protection against tumors and mortality. Thus, chickens may be potentially infected with both vaccine and virulent MHV-1 strains. In this study, a multi-qPCR method was developed in which specific and sensitive detection of simultaneous, virulent MHV-1, CVI988, HVT and chicken DNA was validated with reference strains and organs collected from commercial / domestic poultry enterprises. It is thought that multi-qPCR method will provide more advantages in diagnosis because of its ease of use, reliability, technically simple, full automation, specific, sensitive and fast.

Keywords: Marek, qPCR, Chicken

O07 Avian Pathogenic *Esherichia coli* (APEC) Detection from Poultry in Turkey

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Abstract

Avian pathogenic *Escherichia coli* (APEC) is considered a significant pathogen for the poultry industry, due to economic losses associated with chronic respiratory disease, septicemia, salpingitis, omphalitis, and embryo death. The prevalence of APEC in the poultry in Turkey is still relatively unknown and assessment of pathogenesis is limited. The aim of the current study was to evaluate the occurrence and frequency of APEC virulence associated genes from poultry flocks in Midwest of Turkey. For this study, 72 APEC isolates were recovered from the internal organs of broilers with clinical signs of colibacillosis. A 9 gene multiplex PCR protocol was used to assess the presence of plasmid and chromosomal encoded virulence genes *cvaC (*679 bp)*, iroN2 (*553 bp)*, ompT (*496 bp)*, hlyF2 (*450 bp)*, etsB (*380 bp)*, iss (*323 bp)*, aerJ (*302 bp)*, ireA (*254 bp) and *papC (*205 bp). Resistance patterns against 15 antibiotics were also determined using the Sensititre NARMS panel for 72 APEC isolates which includes the same virulence genes. The results of multiplex PCR found that 54.16% of the examined isolates harbored at least one of the tested virulence genes. Prevalence levels of the genes *iro*N (30.55%), *omp*T (36,11%), *hly*F2(36,11%), *est*B(18.05%), *iss* (33.33%), *aer*J (36,11%), *ire*A (12,5%) and *pap*C (6,94%) were detected from the isolates as the most prevalent genes for APEC. Data suggests that some of the strains harbor a virulence plasmid. We further established that ampicillin, ciprofloxacin, sulphamethaxazole/trimethoprim and sulfisoxazole were the most frequently observed resistance traits while greatest susceptibility was observed for azithromycin, cefoxitin, ceftiofur, and ceftriazone.

Keywords: APEC, antimicrobial resistance, virulence genes, poultry.

IS06 Importance of PLF Technology in Broiler Production; Lameness Detection by Image Processing and Individual Feed Intake Measurement by Sound Technology

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Abstract

Based on the constantly growing world population and the increasing income of countries such as Brazil, Russia, India and China, which are called BRICS countries, demand for meat and animal products worldwide is expected to increase by at least 50% over the next 20 years. The first question that comes to mind is how to achieve high-quality, sustainable and safe meat production that can meet this demand. As a response to this question, intensive animal husbandry systems are implemented but they are faced with serious problems. Along with the growing number of animals in farms, concerns about food safety and animal health are also increasing. At the same time, the environmental impact of the livestock sector remains an important issue. Finally, it is necessary to question how the farmer, who is the main figure in this process, can overcome the problems brought about by this system while carrying out intensive livestock production and how it can lead a life. Because, in previous years, farmers were able to control the health and welfare of animals through visual and auditory observations, however nowadays it has become impossible to realize this because of the increasing number of animals and the increase in administrative and logistic workloads. Therefore, the farmers must be supported by technology to detect any problems in time and take prevention in regarding to the sustainability of intensive livestock farming. Unlike the previous approaches, sensitive livestock systems are a new management system that focuses on improving the life of animals, providing a real-time monitoring and control system, giving immediate warning if problems arise, in order to be taken immediate actions by farmers. The main purpose of this system is not to replace farmers, veterinarians or zootechnicians, but to support them as their eyes and ears. Precision livestock technologies provide unlimited monitoring time, because farmers get tired and sleep, but computers and technological devices do not get tired and need no rest. The Food and Agriculture Organization of the United Nations (FAO) stated that technology solutions in agriculture and livestock production systems will play a key role in ensuring adequate food supply for the expected 9.7 billion population by 2050. It is clear that precision livestock technologies will have a very important and positive effect in the work of farmers engaged in the production of broiler chickens, and may be of particular interest to young farmers and farmer candidates. These new technologies, which are not known and used sufficiently by our farmers who are engaged in broiler production in our country, are being developed in line with the needs of our country's farmers is essential in terms of sustainable intensive broiler production. Therefore, this challenging approach of PLF should be transposed to practice in order to further develop our broiler sector and compete with other countries in the world. However, this is only possible when teams composed of different research fields, like physiology, zoology, and technology. In a word, to achieve this, "technology science" and "animal science" need to collaborate!

Keywords: Precision livestock farming, image processing, sound analysis, feed intake, lameness

O[08] Advances in Gel Delivery Technologies for Hatcheries

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Abstract

Subject: Recent improvements in edible Gel droplet technologies have made it commercially applicable in hatcheries, generated earlier and stronger coccidiosis vaccine cycling and protection than aerosol or feed spray applications, and are adaptable for other enteric vaccine, respiratory vaccines, and competitive exclusion additives.

Problem. Veterinarians seeking earlier disease prevention and more control over animal health applications have pushed to perform more vaccinations in hatcheries. Edible gel droplets administered to day-old chicks was proposed as a potentially effective delivery method for coccidiosis vaccine, but it lacked a widespread, commercially relevant means to prepare and administer the gel. Consequently, delayed coccidiosis vaccination in feed remains a common technique, as does spraying by aerosol in the hatchery, a less-effective relic adapted from respiratory virus vaccination techniques. This work describes new advances in gel preparation and convenient dosing systems to effect earlier and more protective edible coccidiosis vaccine delivery, with a view to permit eventual combination with other enteric treatments such as *Salmonella* vaccine, competitive exclusion microbials, and respiratory antigens in the same gel.

Materials and Methods

This work evaluated commercial coccidiosis vaccine applied via an edible gel spray, prepared using hatchery tap water, compared to spray vaccination via the feed or aerosol distilled water spray on d1. The first study compared day of hatch coccidiosis vaccine administered to chickens (n=60), sprayed in gel (GL) or sprayed on feed (FD), assessing the oocyst excretion pattern in feces of broilers under battery conditions after vaccination on d1. Chickens were challenged on d20 with *Eimeria* spp. isolated from broilers in the field to compare vaccine protection and impact on growth performance. A second study compared the same coccidiosis vaccine in hatchery GL to aerosol spray (AS). Commercial layer chicks (n=40) were vaccinated on d1 by either GL or AS, and OPG surveyed through d30 to compare vaccine early cycling patterns.

Results and Discussion

Study 1 vaccine cycling patterns revealed earlier and more numerous oocyst excretion per gram of feces (OPG) using GL rather than FD application. Chicks vaccinated on d1 began excreting detectable OPG on day 7 for GL compared to day 8 for FD. The OPG in the first cycling wave from d7-11 were greater for those vaccinated via GL than via FD. That pattern of amplified vaccine cycling persisted, with the vaccine OPG from GL exceeding that of FD on all days except d20. The GL vaccine was also more uniform among the birds than FD vaccine, indicated by a greater proportion of GL fecal samples being positive for vaccine oocysts surveyed on

d9 (GL 53%, FD 7%), d14 (GL 67%, FD 7%), and d19 (GL 93%, FD 60%). Exhibiting a compounding effect, the more positive GL fecal samples also contained more vaccine oocysts than the corresponding FD vaccine (oocyst density scores, range 0-3). Scores were d9 (GL 0.53, FD 0.07), d14 (GL 0.67, FD 0.13), and d19 (GL 1.60, FD 0.80).

Intestinal lesion scoring (ILS), after challenging on d20 and euthanizing on d27, reflected the severity of infection. Non vaccinated controls (NV) exhibited significantly higher ILS at 3.71, than non-challenged controls (NC) at 1.29 ($P<0.05$). FD did not protect birds from the effect of the challenge, the FD ILS at 3.37 being not significantly different than NV birds. GL, in contrast, significantly protected birds, with GL ILS reduced to 2.86 ($P<0.05$ versus NV). The improved ILS attributed to GL was paralleled by fewer infectious challenge organisms shed. Mean infectious coccidia OPG 7d after challenge numbered: NV 313,421, FD 85,991, and GL 38,189. The stronger GL vaccination did not harm feed conversion or growth rate, as there were no significant differences between GL and FD.

Study 2 OPG cycling patterns found oocysts appearing on d7 in GL fecal samples but not AS. AS oocysts appearing on d8, measured 9,000 OPG, compared to GL 48,400. That GL advantage continued on d9, with FD OPG reducing to 3,600 and GL to 41,800, maintaining a benefit of earlier vaccine cycling for GL. AS does not present enteric coccidiosis vaccine in a manner that facilitates as much consumption and therefore first-cycle vaccine uptake as to GL.

Conclusion

Hatcheries work to remain efficiency while performing ever-more complex responsibilities. Increasing the number of vaccinations and oral treatments in the hatchery prompts veterinarians to combine them into single applications, where possible. This work indicates gel is better for edible vaccines, and subsequent investigations have expanded gel application to other treatments including *Salmonella* vaccine, respiratory vaccines, and competitive exclusion microbes in various combinations.

O09 Production of a Functional Chicken Meat Product Using 3D Food Printer

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Abstract

Three-Dimensional (3D) printing has emerged as a very suitable technology to address the challenges in designing foods for special needs. This technology consists of overlapping layers of food ingredients following an algorithm by a device which is programmed for each food product. In recent years, 3D food printers are gaining interest within the area of meat science because it can provide consumers with new and exciting alternatives to attain high quality, functional meat-based products; to develop formulations and structural characteristics for the elderly; as well as for various types of patients, including children, who require special nutrition. In the present study, the aim was to develop a new functional chicken meat product with enhanced nutritional value and flavor using 3D food printing technology, thus, to extend the poultry product range using an emerging technology. A new cartridge content was developed using an elaborated chicken meat product formulation with the addition of starch at three different concentrations (1.0%, 2.5%, and 5.0%, w/w) to obtain good quality printing parameters. The best result was obtained in the group with the addition of 2.5% starch in terms of printability properties. The results from this study provide a basis for future research on 3D food printing technology to develop food products with desirable flavor, shape and size for customized food fabrication.

Key words: 3D food printing, 3D food printer, Personal food design, Functional chicken meat product

O[10] Effect of In Ovo (IO) Leptin Injection on Leptin Hormone Level in the Egg Yolk Sac and Hatching Performance

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Abstract

The aim of this study was to investigate the effect of in ovo (IO) leptin injection on leptin hormone level in the egg yolk sac and hatching performance. A total of 408 eggs obtained from Ross 308 broiler breeders were used. The eggs were weighed and placed in incubator as the non-injected group control (C) and 3 injected groups; 100 µl of phosphate buffer solution (PBS) group; 0.5 µl leptin in 100 µl of PBS ($L_{0.5}$) group; 1 µl leptin in 100 µl of PBS (L_1) group (100 eggs/ 4 replication /group) which were incubated at 37.8 °C and 65 % relative humidity. Leptin hormone levels in the egg yolk at onset of incubation, yolk sac and residual yolk sac at hatching, eggshell temperature, rectal temperature, egg water loss, and variables of hatching performance were measured. The pure leptin hormone (Rat leptin-Sigma-Aldrich, USA) dissolved in PBS as 0.5 ve 1 µl leptin hormone was IO injected to yolk sac of embryo at day 7 of incubation. The highest leptin hormone level in the egg yolk sac was measured as 754.17 ng/ml on the 7 day of incubation. At the onset of incubation, egg yolk leptin level was found to be statistically higher than that in the yolk sac on the 15 day of incubation (P<0.001). On the 7 day of incubation, eggshell temperature of the $L_{0.5}$ group was higher than that of the L_1 and C groups, but eggshell temperature of the $L_{0.5}$ group was found to be lower than other groups on the 11 day of incubation. On the 19 day of incubation, eggshell temperatures in all injection groups were higher than C group. External pipping time was similar in the IO leptin injection groups to C group, but hatching time was shorter in the IO leptin injection than C group. No significant effect of IO leptin injection was found on yolk leptin level, egg water loss, rectal temperature, hatching rate and embryonic mortalities. The results of this study indicated that the effect of IO leptin administration was shortened total incubation duration and embryonic age-related was changed yolk/yolk sac leptin level.

Key words: İn ovo enjeksiyon, leptin, sarı kese, embriyo

IS07 Novel Protein Sources in Poultry Nutrition

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Abstract

It is expected that the need for protein sources for application in feed will increase drastically in the coming decades. This paper addresses the background of this increased protein demand, and the perspectives of some novel plant proteins for poultry feed. In particular the protein providing or replacing properties of macro algae (seaweeds), micro algae, leaf proteins and free amino acids will be discussed. The chemical composition of seaweeds shows large variation due to among others species, location, and harvesting season, which provides both opportunities and challenges. The high mineral content and potentially high heavy metal content requires attention. There is limited and inconsistent information regarding the nutritive value of seaweeds for poultry. Some intact seaweeds showed prebiotic effects or reduced the bacterial load in case of a bacterial challenge. Recent *in vitro* studies with extracted seaweed polysaccharides demonstrated anti-bacterial effects, as well as stimulating effects on the immune system, energy metabolism and oxidative response. These seaweed properties seem to be very promising, but *in vivo* poultry studies should be conducted to validate these findings. Based on results available from literature it can be concluded that micro algae from a nutritional point of view can be considered as a useful protein source in laying hen and broiler diets. Inclusion levels between 5 and 15% seem to be applicable without negative effects on bird performance. The effects of cell wall disruption techniques on nutrient digestibility need further investigation. Moreover, there are several indications that micro algae contain bioactive components, which might contribute to an improvement of the quality of meat and eggs and to bird health. Besides, micro algae potentially have a very high protein yield per hectare, making algae a promising novel protein source. Intact grass might to some extent contribute to the protein supply of poultry. The fibre content of intact grass, however, limits its use in poultry diets. Biorefinery might increase the possibilities to use grass and other leaves by separating the protein and fibre fractions. In vivo studies are required to demonstrate the nutritional value of extracted leaf proteins in poultry. Supplementation of free amino acids to the diet allows a substantial reduction of the soybean meal content of broiler diets, thereby reducing the crude protein content, without adverse effects on growth performance and slaughter yield, provided that diets are supplemented with sufficient lysine, and free methionine, threonine, arginine, isoleucine, valine and glycine in the recommended ratio to lysine. Based on the current nutritional knowledge we conclude that micro algae and free amino acids currently already can substantially contribute to poultry diets as novel proteins or protein replacers, whereas the protein value of seaweeds and leaf proteins for poultry needs further investigation.

Introduction

Developments in protein demand

The global demand for animal source food is expected to increase over 50% and 70% until 2030 and 2050, respectively, compared to 2000, because of growth of the world population, increased incomes, and urbanization, mostly in developing regions [1, 2]. As a consequence, the demand for animal feed will increase as well. Currently, global feed production is already rapidly increasing, as from 645 Mton in 2005 to 1032 Mton in 2016 [3], with Asia and Africa being the fast growing regions. It is prospected that the global demand for feed will amount 1500 Mton in 2050, which is a real challenge, especially with respect to the demand for dietary protein. Currently, major parts of the world are not self-sufficient in protein supply. In the EU, for instance, the rate of self-sufficiency of protein for feed currently amounts 42% [4], while the deficit of soybean in China increased linearly over the last decade, from 29 to 91 Mton of soybean [5].

How to fulfil the increased protein demand?

It need to be investigated which options are available to produce such a large amount of feed, and in particular feed proteins in the coming decades. Basically, the following strategies can be applied: i) use of fallow land, ii) increase of the protein yield per hectare of the currently used crops, iii) improvement of the feed and protein efficiency of farm animals, iv) prevention of waste of resources, e.g. by closing nutrient cycles, and v) focus on the development of novel protein sources. The FAO expects that up to 2050 the increase of arable land will only amount 5% [2], indicating that the contribution of the first strategy to the feed protein supply seems to be limited. This paper focuses on some novel protein sources. Table 1 provides an overview of the protein yield per hectare of different crops cultivated under EU conditions.

From Table 1, it can be concluded that replacement of wheat by soybean currently will not increase the protein yield per hectare. In contrast, cultivation of legumes and grass can contribute to an increased protein production. Cultivation of aquatic proteins, like duckweed, micro and macro algae are very interesting because of high protein content (duckweed and several micro-algae) and very high yields, as has been demonstrated in pilot experiments. Not only the high yield per hectare, as for duckweed, and the high protein level are interesting, but also the fact that these new putative protein sources do not need high quality arable land for cultivation. However, large scale cultivation, processing and potential application as feed of these novel plant proteins still needs more research [6]. Besides aquatic proteins, also extracted grass protein and increased usage of free amino acids might contribute to the protein supply of poultry.

The aim of this paper is to address the nutritional value of some novel plant proteins for poultry and to consider the contribution of free amino acids to the protein supply of broilers.

Table 1. Protein content, DM yield, and protein yield of various protein crops cultivated under EU conditions [6]

	Protein content (% in DM)	DM Yield (ton DM/ha/y)	Protein yield (ton protein/ha/y)
Oil seeds – soybean	40	1.5-3	0.6-1.2
Oil seeds – rapeseed	25	3	0.75
Oil seeds – sunflower	23	3	0.7
Legumes (pulses) – peas/beans/ lupine	17-35	4-6	1-2
Legumes (forage) – lucerne	19	13	2.5
Cereals – oat	12-15	3-5	0.4-0.75
Pseudo cereals – quinoa	12-18	3	0.4-0.5
Leaves – grass	12	10-15	1.2-2
Leaves – (e.g. sugar beet leaves)	12	4.5	0.5
Macro algae - seaweed	10-30	25	2.5-7.5
Micro algae	25-50	15-30	4-15
Duckweed	35-45	30-40	10-18
Wheat (as reference)	11	10	1.1

Nutritional Value of Novel Proteins For Poultry

Seaweeds

In the previous centuries, naturally grown seaweed was used as an ingredient in the diet of e.g. poultry in coastal regions of Europe (e.g. Norway, Scotland, France) [7]. The historical use of seaweed has been documented in recent reviews [8-10]. In the present large scale commercial animal production, seaweed is not used to any significant extend. Adequate scientific insight in de nutritive value of seaweed is lacking and the number of published animal studies using intact seaweed is limited. Because of the scarcity of agricultural land, the extension of marine production by large scale cultivation of seaweed could significantly contribute to biomass production with potential use as poultry feed ingredient, intact or as residue from biorefinery processes, as a source of macro nutrients.

The chemical composition of seaweeds, and consequently their nutritional value, varies substantially, depending on e.g. seaweed species, origin, and harvesting season [11]. Results from our institute [12] also indicated large variation in nutritive value between seaweed species (e.g. crude protein 56-311 g, fat 7-35 g, crude fibre 28-88 g, starch 1-90 g/kg DM), and relevant similarities within species from different locations of origin (Table 2). The high contents of non-starch polysaccharides (255-622 g/kg DM), ash (173-445 g per kg DM), potassium, sodium, and heavy metals in some intact seaweed samples is of concern and attention is required in case of application in poultry diets. Mean in vitro ileal (46-80%) and total tract (69-89%) dry matter digestibility varied drastically, all being lower than the values obtained for soybean meal as reference ingredient (84 and 98% ileal and total tract) in the Boisen vitro system [12]. In our institute, a broiler digestibility study with seaweeds was conducted showing that the precaecal crude protein digestibility of Saccharina silage and Saccharina silage residue amounted 65.9 and 69.4%, respectively (Van Krimpen, personal communication).

Table 2. Chemical composition of selected seaweeds on a dry matter basis

	g/kg	g/kg DM											
Seaweed species[1]	DM[2]	ASH	OM[3]	CP	CFAT	CFIBRE	SUGAR	STARCH	NSP[4]	NDF	ADF	ADL	HCl-ASH
Laminaria digitata (S)	891.1	275	725	115	16	73	2	2	517	120	200	36	7
Laminaria (I)	923.2	367	633	102	11	69	1	1	449	91	164	28	7
Saccharina latissima (S)	873.8	243	757	92	10	71	6	1	576	122	185	23	11
Saccharina latissima (F)	902.1	273	727	146	12	62	1	1	505	96	171	7	6
Ascophyllum nodosum (S)	882.9	214	786	56	38	54	30	1	606	162	331	180	3
Ascophyllum nodosum (I)	910.1	411	589	71	10	88	1	1	417	152	298	48	11
Palmaria palmata (S)	938.9	209	791	176	12	35	35	17	516	312	50	6	7
Palmaria palmata (F)	948.7	228	772	167	13	28	48	22	492	347	42	5	4
Chondrus crispus (S)	883.1	176	824	123	17	31	6	26	622	392	40	9	2
Chondrus crispus (I)	898.8	445	555	156	7	45	3	90	255	190	53	14	152
Ulva lactuca (S)	841.6	243	757	87	23	76	24	75	472	385	141	70	20
Ulva lactuca (F)	879.7	260	740	209	35	76	12	73	334	329	143	69	8
Ulva lactuca (I)	883.2	173	827	311	21	57	7	42	389	259	135	69	11

OM = organic matter, CP = crude protein (N * 6.25), CFAT = crude fat, CFIBRE = crude fibre. Each value in the table is based on one analysis in duplicate.

[1]S = Scotland, I = Ireland, F = France. [2]DM content of the dried product.

[3] Calculated as 1000 – Ash. [4] Calculated as 1000 – Ash – Crude protein – Crude fat – Starch – Sugars.

In chickens, only relatively low levels of intact seaweed have been included without negative effect on performance characteristics. Ventura et al. [13] determined a very low energy value of the green seaweed *Ulva* rigida, and a negative effect on feed intake and growth performance at an inclusion rate of 10% or higher. Similarly, addition of 10 – 15% *Ascophyllum* nodosum meal to poultry feed induced diarrhoea in the birds, whereas an addition up to 7% had no negative effects [14]. El-Deek and Brikaa [15] determined a relatively high energy value of dried red seaweed (*Polysiphonia* SPP) in poultry and demonstrated that up to 3% did not adversely influence growth performance of ducks. El-Deek and Al-Harthi [15] did not observe a negative effect of up to 12% inclusion of *Sargassum* meal on feed utilisation and egg production in laying hens whereas El-Deek et al. [16] observed a significant decrease in growth performance and feed utilisation in broilers with incremental inclusion levels of *Sargassum* spp. up to 6%. Abudabos et al. [17] did not observe a negative effect on growth performance of the inclusion of 3% Ulva lactuca in broiler diets at the expense of maize. The majority of studies cited above included intact seaweed, generally sun-dried and ground, into the diet. Recently, we argued that the costs of seaweed production presumably remain too high for large scale inclusion of the intact product in animal diets [18]. It may be economically more feasible to optimize the use of seaweed with biorefinery processes and use specific fractions or residues for inclusion in animal diets. High value fractions may be extracted and used for applications in food processing, pharmaceuticals, chemical engineering and cosmetics [11]. Until now this approach has received little attention. Recent work in our lab with intact *Ulva* lactuca and an extracted fraction, remaining after enzyme treatment and centrifugation to use the soluble carbohydrates as energy source, indicated a higher in vitro protein and organic matter digestibility of the extracted fraction [19]. These results indicate that the value of feed ingredients, derived as co-product from biorefinery processes may be promising, but largely dependent on the used seaweed species, the processes involved, and the composition of the extracted and residue fractions.

In addition to the nutritive properties, seaweeds may also contain biologically active compounds with a large panel of applications in industries such as pharmaceuticals, cosmetics, food and animal feed. Seaweeds may contain relatively high amounts of polysaccharides, e.g. alginate, carrageenan, agar, fucoidan, laminarin, mannitol, and ulvan, which have bioactive properties. These polysaccharides have shown to exert anticoagulant, antiviral and antibacterial, anti-proliferative and immuno-modulatory properties [20-23].

Part of these properties were confirmed in an in-vitro study conducted in our institute. In this study the effects of polysaccharides from *Saccharomyces cerevisiae* containing β-glucan and mannan (SC, positive control), micro algae containing β-glucan (MA), brown macro algae containing fucoidan and laminarin (BA) and green macro algae containing ulvan (GA) on gene expression in intestinal porcine jejunum epithelial cells (IPEC-J2) were investigated in the presence and absence of the enterotoxigenic bacterium *Escherichia coli* k99 strain (ETEC) as an *in vitro* challenge. Gene expression was measured in IPEC-J2 cells after 2 and 6 hours of incubation using “whole genome” porcine microarrays. Analysis of the generated transcriptomics datasets using bioinformatics programs indicated that 18 acute phase proteins and 49 pathways involved in immune response were modulated in the IPEC-J2 cells by the polysaccharides in the presence of ETEC. Without ETEC challenge, algae modulated 3 (BA) to 13 (MA) pathways involved in immune response, versus 1 with SC. With challenge, algae modulated 18 (GA) to 36 (BA) pathways involved in immune response, versus 13 with SC. Other affected pathways included energy metabolism, intestinal carriers and oxidative response. In this study, the gene expression of acute phase proteins and regulation of pathways involved in the immune response, energy metabolism and oxidative response were modulated more with brown, and green macro algae and with micro algae than with SC at similar inclusion level. These new data may suggest that some NSPs from algae may be more effective than NSPs from yeast to alert the immune system in case of infection by E. coli. Berri et al [24] also observed a higher mRNA expression of gut immune response mediators in an in vitro system of differentiated porcine intestinal epithelial cells (IPEC-1), because of the use of an aqueous marine-sulphated polysaccharide (MSP) extract, prepared from the green seaweed *Ulva armoricana*. Moreover, this MSP was tested as an antibacterial compound against 42 bacterial strains and isolates found in livestock animals, showing that growth of both Gram-positive and Gram-negative bacteria was affected. In a laying hen study, 2 red seaweeds (*Chondrus crispus* and *Sarcodiotheca gaudichaudii,* 0.5%, 1% or 2% inclusion level) showed prebiotic effects, as indicated by a higher abundance of beneficial bacteria in the ceca, e.g. *bifido* bacteria, and a lower abundance of *Clostridium perfringens* [25]. Moreover, villus height and villus surface area increased as well. Feeding 0, 500 or 1000 ppm of an *Ascophylum nodosum* extract to challenged young broilers resulted in a reduction in *Campylobacter jejuni* colonisation, while a higher expression of tight-junction genes was observed [26].

In summary, the chemical composition of seaweeds showed large variation due to among others species, location, and harvesting season, which provides both opportunities and challenges. The high mineral content and sometimes high heavy metal content requires attention. There is limited and variable information regarding the nutritive value of seaweeds for poultry. Some intact seaweeds showed prebiotic effects or reduced the bacterial load in case of a bacterial challenge. Recent *in vitro* studies with extracted seaweed polysaccharides demonstrated anti-bacterial effects, as well as stimulating effects on the immune system, energy metabolism and oxidative response. These seaweed properties seem to be very promising, but *in vivo* poultry studies should be conducted to validate these findings.

Micro Algae

Spirulina (blue-green alga), and Chlorella (green alga) are the most prominent protein-rich algae, which are commercially produced. On a dry matter basis, protein content of *Chlorella vulgaris* and *Spirulina* ranged from 51 to 58% and from 60-71%, respectively [27]. The most dominant minerals in the ash fraction are sulphur, potassium, sodium and chloride, and these mineral contents have to be taken into account during feed formulation. Amino acid composition showed a lower concentration of lysine, histidine and phenylalanine in proteins of the Spirulina meal than in protein of soybean meal, whereas the other essential amino acids were present in higher concentrations. In general, protein digestibility was found to vary between 55 and 82%, depending on algae species and method of drying the algae [28]. The *in vitro* crude protein digestibility of a mixture of *Chlorella sorokiana* and *Scenedesmus obliquus* (52.5% crude protein and 7.0% crude fat), determined in our lab, was 50% [29]. In a broiler study, the digestibility of *Spirulina platensis* (58.2% crude protein, 2.6% crude fat) was investigated and compared to soybean meal [30]. The amino acid profile relative to lysine of this micro algae was comparable to the profile of the reference soybean meal. Protein and gross energy digestibility of the *Spirulina*, however were moderate with values of 52.9% and 56.1% respectively. A dried *Spirulina sp*. (76.0% crude protein, 4.95% crude fat was used in studies of Evans et al. [31] and Boney et al. [32] to determine amino acid digestibility and performance in broilers. True amino acid digestibility was high with coefficients ranging between 90 and 95% [32]. Dietary supplementation of this micro algae up to 16% did not affect broiler performance (3-21 d of age), but feed intake and body weight gain were reduced in birds fed a diet with 21% of this *Spirulina* [31]. Some micro algae have a very thick and tough cell wall, which hampers the digestibility of the nutrients inside the cells. Therefore, application of mechanical (e.g. bead milling or ultra-sonification) or non-mechanical (e.g. chemicals or enzymes) cell wall disruption methods might increase protein digestibility. More research is required to determine the effect of cell wall disruption on nutrient digestibility of micro algae. Inclusion of 1% dried *Chlorella vulgaris* to a broiler diet improved body weight gain with 3.5% (1549 vs. 1603 g) and feed conversion ratio with 8.5% (1.52 vs. 1.66) [33]. The effect of inclusion of sun dried *Spirulina platensis* (62.5% crude protein, 3.0% crude fat) in a commercial broiler diet was examined in a 12 week study, in which diet fish meal or peanut cake was replaced by algae in concentrations of 140 and 170 g/kg [34]. Based on a similar feed efficiency, protein conversion rate, and the extent of carcass lean meat content, it was concluded that substitution of fish meal or peanut cake by algae did not affect broiler performance. Moreover, none of the diets affected body composition and histopathology of the various organs of the broilers. Meat quality remained also unchanged. Sensory evaluation of the meat by a test panel showed that broilers fed on the algae-based diets yielded better meat in terms of texture, flavor and color. In summary, a dietary inclusion level of about 5-10% of intact algae can be safely used as partial replacer of conventional protein sources of the poultry diet, while higher concentrations might in the long-term cause adverse effects, like a less efficient feed conversion and a decreased protein and energy efficiency.

In laying hens, no differences were observed in egg production and egg quality (size, weight, shell thickness, solid content of the egg, albumin index, etc.), and feed conversion ratio between hens fed diets containing 12% *Chlorella* (cultivated on effluent) and hens fed the control group [35]. In a study of Ekmay et al. [36], a defatted protein rich green (*Desmodesmus spp*, 31.2% crude protein, 1.5% crude fat) and a full-fatted micro algae (*Staurosira sp*, 13.9% crude protein, 9.3% crude fat) were included in laying hen diets at 25.0 and 11.7%, respectively and fed from 26 to 40 wk of age. Despite a lower feed intake, final body weight and bird performance were not affected by the micro algae.

Besides their protein providing properties, micro algae have several other functional properties. They are able to enrich poultry meat or eggs with omega-3 fatty acids [37, 38]. Moreover, it has been shown that feeding *Spirulina platensis* to broilers challenged with sheep red blood cells upregulated macrophage phagocytic activity as well as metabolic pathways leading to increased nitric oxide synthase activity [39]. It was also demonstrated that *Spirulina* supplementation increased several immunological functions implying that a dietary inclusion of *Spirulina* at a level of 1% may enhance disease resistance potential in broilers and layer pullets [40]. Recently, we conducted an in-vitro study in our institute, in which the effects of *Spirulina, Heamatococcus, Chlorella,* and a mixture of *Chlorella sorokiana* and *Scenedesmus obliquus* on gene expression in intestinal porcine epithelial cells J2 (IPEC-J2) were investigated in the presence and absence of the enterotoxigenic bacterium *Escherichia coli* k99 strain (ETEC) as an *in vitro* challenge. Gene expression was measured in IPEC-J2 cells after 2 and 6 hours of incubation using “whole genome” porcine microarrays. The micro algae effected expression of genes involved in many biological functions, e.g. T cell development, leukocyte recruitment, inflammatory response mediation, and protecting oxidative stress (Hulst, personal communication). These health improving properties are due to presence of anti-bacterial components (e.g. chlorellin), anti-viral components (e.g. spirulan) and anti-oxidants in micro algae [41].

Based on results available from literature it can be concluded that micro algae from a nutritional point of view can be considered as a useful protein source in laying hen and broiler diets. Inclusion levels between 5 and 15% seem to be applicable without negative effects on birds' performance. The effects of cell wall disruption techniques on nutrient digestibility need further investigation. Moreover, there are several indications that micro algae contain bioactive components which might contribute to an improvement of bird health and the quality of meat and eggs. Besides, the potential protein yield per hectare of micro algae is very high, making algae a promising novel protein source. The present costs price of micro algae, produced in large scale algae plants, however, is estimated to range between € 4.50 – 6.30 per kg DM, depending on the cultivation system (http://www.enalgae.eu/). Therefore, the price of micro algae is currently not competitive as a protein source.

Leaf proteins

In terms of protein yield per hectare, grass cultivation is very efficient in North-West Europe (Van Krimpen et al., 2013). Whole grass is a common ingredient in organic poultry husbandry, and access to a grass-clover pasture can substantially contribute to the protein supply of the broilers. Broilers were able to realize 7% of the recommended amount of protein by the intake of grass-clover from the pasture [42]. Laying hens are able to consume considerable amounts of fresh grass, which might contribute for 12 – 13% of the total dry matter intake [43].

Protein content and digestibility of fresh grass depend on a number of factors, among others stage at harvest. Protein content of fresh grass, harvested at either a young or older (3 wk later) stage, was 148 and 108 g/kg DM [44]. Van der Peet-Schwering et al. [45] observed that protein digestibility of grass silage in sows depended on grass yield/ha and ranged from 40% at a yield level of 5 ton dm/ha to 63% at 1.8 ton dm/ha. The protein content decreased and the fibre content increased with increasing stage of growth and related grass yield/ha. Therefore, extraction of protein from grass, thereby separating proteins from fibres, might increase their applicability in poultry diets [46]. These proteins can be valorized as alternatives to extracted soybeans [47].

In recent years, grass processing techniques have been developed. Zhang et al. [48] developed a cost-effective protein separation technique from leaves, based on alkaline extraction. Depending on temperature, amount of NaOH, and extraction time, up to 95% of total protein could be extracted, with a protein content of the extract up to 52%. Pre-treatment, using ethanol or enzymes reduced the alkali consumption by 25% and further improved protein extraction yield and purity [49]. Currently, mobile grass biorefinery equipment is available to produce grass protein on a semi-commercial scale (http://grassa.nl/). By this, grass is fractionated in different fractions, e.g. a fibre-low/protein-rich fraction (50% crude protein on a dry matter base), a fibre-rich fraction, and a sugar-rich fraction [50].

Already in 1965, digestibility of different leaf proteins, e.g. from rye, potato, pea, and red clover leafs, after freeze drying was determined in rats [51]. Protein digestibility was reasonable, ranging from 70.6% in red clover leaf protein to 84.8% in rape leaf protein. Until now, however, hardly any information regarding nutritional value of grass proteins for poultry is available. *In vitro* digestibility studies in our lab showed that the dry matter digestibility increased from 50 (intact grass) to over 90% (extracted grass protein), suggesting that extracted grass-protein can be an interesting protein source for (organic-housed) monogastrics (Van Krimpen, personal communication).

It can be concluded that intact grass to some extent can contribute to the protein supply of poultry. The fibre content of intact grass, however, limits its use in poultry diets. Biorefinery can improve possibilities to use grass and other leaves by separating the protein and fibre fractions. *In vivo* studies are required to determine the nutritional value of extracted leaf proteins in poultry.

Free amino acids

Use of broiler diets with a reduced crude protein (CP) content and supplemented with increased levels of free amino acids (AA) to cover the AA requirements would be helpful in reducing the use of imported soybean meal of South American origin. Reducing the crude protein content of broiler diets, however, increases the risk of a lower growth performance, when (semi-) essential amino acids become limiting. In order to reduce crude protein in broiler feeds while maintaining growth performance, it is essential to keep the supply and balance of limiting amino acids in line with the broilers requirements by adequate dietary supplementation of free amino acids. Published studies show that, low protein diets allow to achieve maximum level of performance, provided that the digestible lysine content and the ratio of other essential amino acids to lysine are maintained [52, 53]. However, in low protein diets glycine and serine, that are currently considered as non-essential amino acids [52], can become limiting, either because there are not enough metabolic precursors for the respective amino acids available, or because endogenous metabolization processes are too slow [54, 55]. This could well be the reason why several researchers found decreased performance results with low protein diets supplemented with amino acids, without maintaining the glycine + serine to lysine ratio [56-58].

When reducing the crude protein content , glycine and serine levels are reduced as well [52]. Therefore, marginal levels of dietary glycine and serine may be the reason for a decrease in performance of broilers when feeding low protein diets, even when they are supplemented with essential amino acids. Several studies showed that glycine supplementation prevent adverse effects on broiler performance when low protein diets were provided [59-61]. In a study in our institute with broiler chickens from 8–35 days of age, a lower growth performance of broilers fed low protein diets was observed, despite essential amino acids (lysine, methionine, tryptophan, threonine, arginine and isoleucine) were supplemented to 10% above CVB [62] requirements (Veldkamp, personal communication). The authors suggested that the lack of glycine and serine might explain the reduced growth performance in low protein diets. Because of the importance of glycine in low-protein diets, it is considered as the fourth limiting amino acid after lysine, methionine and threonine [63]. Also according to Ospina-Rojas [61], glycine is a limiting amino acid in low protein diets, especially in the starter phase but also thereafter. Based on the results in the study of Veldkamp, personal communication) and in literature, it seems to be essential to add free glycine to low protein diets, to maintain the production results of broilers. We conducted a broiler study in which the effects of low CP diets, with partial replacement of soybean meal by free AA, on animal performance, slaughter yields, litter quality, footpad lesions, economic performance and the ecological footprint were evaluated. In this study, dietary soybean meal content in the grower diets was reduced from 27.3% (control) to 17.3% (-3% CP), while soybean meal content in the finisher diets was reduced from 25.0% (control) to 14.6% (-3% CP) [64]. The birds that were fed the low CP diets had similar or even better growth performance as broilers fed the control diets. The best overall performance was obtained with the CP-2% diet program. Broilers fed the -2% CP or -3% CP diet program had a significantly improved feed conversion ratio. Broilers fed the low protein diets had a lower water intake, a better litter quality and less severe footpad lesions compared to broilers fed the control diets. The use of broilers diets with an up to 3% lower CP content while maintaining dietary concentrations of essential AA hardly influenced the slaughter yields. Only the breast meat yield (as % of the carcass) of broilers fed the diet with 3% lower CP content was lower, while the breast meat weight did not differ. Overall it can be concluded that supplementation of free AA to the diet allows an absolute reduction of the soybean meal content of broiler diets by 10%, which is a relative reduction of 40%, thereby reducing crude protein content, without adverse effects on growth performance and slaughter yield provided that diets are supplemented with adequate lysine, and with free methionine, threonine, arginine, isoleucine, valine and glycine in the recommended ratio to lysine.

Conclusion

The global demand for animal sourced food in 2050 is expected to have increased by 70% compared to the 2000 demand. Possible strategies to meet this demand are i) use of fallow land, ii) increase in the protein yield per hectare of the currently cultivated crops, iii) improvements in feed and protein efficiency of farm animals, iv) prevention of the wasting of resources, e.g. by closing nutrient cycles, and v) focus on the development of novel protein sources. The protein yield per hectare is rather low for oil seeds, average for legumes and leaf plants, and high for aquatic proteins and insects. In general, limited studies are available to demonstrate the nutritional value, and particular the *in vivo* protein digestibility of novel proteins in non-ruminant species. Besides their nutrient providing properties, micro algae and seaweeds might positively contribute to the health status of livestock. Studies with low-protein diets show that up to 40% of the SBM inclusion level can be replaced by free amino acids without compromising animal performance levels.

Based on the current nutritional knowledge we conclude that micro algae and free amino acids currently already can substantially contribute to poultry diets as novel proteins or protein replacers, whereas the potential contribution of seaweeds and leaf proteins needs further investigation.

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O[11] Feed Value of Red Worm (*Eisenia fetida*) and Its Usability as an Alternative Protein Source in Broiler Diets

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Abstract

This review was prepared to discuss feed value of red worm (*Eisenia fetida*) and the possibilities of using in broiler diets. Earthworm meals especially red worm (*Eisenia fetida*) contains high levels of amino acids, fat, mineral, vitamin, mono and polyunsaturated fatty acids. It also has antimicrobial and antioxidants properties. The protein requirement of broiler was usually met soy bean meal, fish meal or poultry meal until recently. But the limited production of this feedstuffs, price increases and prohibiting of chicken slaughtering residues led to the need to alternative protein sources. Previous studies reported that the inclusion of red worm to broiler diets positively affected performance parameters, carcass characteristics, meat quality and sensory scores of meat of broilers. Consequently, red worm seems to be suitable using as an alternative protein source in broiler feeds.

Introduction

The most part of the cost of poultry production is feed expenditures (approximately 70%). In most countries, the poultry industry depends on imported feeds. This leads to an increase in poultry production costs, which ultimately results in an increase in the market price of poultry meat (1). In particular, a significant protein deficit has emerged by prohibiting the use of chicken slaughtering residues as a protein source in broiler feed. This has led feed producers to use soy protein or fish meal. Presently, the protein sources for poultry such as fish meal and soya bean are scarce and expensive. Consequently, poultry industry requires alternative protein sources. The earthworm is part of the poultry diet in nature. It has been shown to form insect and worm of 50% of the stomach content of wild bird species (2). Earthworms can be used as an alternative non-conventional protein source in broiler feeds. Earthworms can grow on a wide range of composting organic materials, from fruit to vegetables to kitchen wastes, rendered fish, and poultry, pigs, and cattle manure, thus being potentially interesting in reducing environmental criticisms by transforming waste invaluable biomass (3).

The earthworm that is especially red worm (*Eisenia fetida*) has a high growth capacity. Red worm (*Eisenia fetida*) is a manure worm, which is used at commercial scale to produce compost for use in agriculture. The hardy nature of this worm can help tolerate wide fluctuation of temperature and humidity. This enables easy culturing of this species. Growth rate is very fast and its duration is 70 days. The mature worm can grow up to 1500 mg of body weight. Mature worm, on the average, produces one cocoon every third day and one to three baby worms emerge from each cocoon on hatching after three to four weeks of incubation. Though temperature tolerance is good, it cannot withstand direct sunlight and temperature. 100 kg of worms can be produced in a ton of animal manure (4).

High-proliferation capacity in facilities that aim to produce worm manure, after a while, leads to high production surplus worms, and this high-quality nutrient has the potential to be used as chicken and fish feeds. The red worm (*Eisenia fetida*) has an important advantage because of its high protein content, ideal amino acid composition, high feed evaluation ability, easy and fast production and the use of a wide range of organic wastes in the production process. Many scientists have reported the possibility of using earthworm meal in broiler feeds (3,5-8). EU Health and Food Safety Commission allowed the use of 7 different insect proteins in aquafeed by the 2017/893 regulation. However, although the earthworm is not included in the allowed insect species, it is promising for the future. Until 2020-2022, it is expected to be approved of the use of processed insect proteins in poultry and pig feeds (9).

Nutrient Composition of Red Worm (*Eisenia fetida*)

Information on the nutrient composition of earthworms is limited, making it complex to include them into feed formulation by nutritionists and farmers. When the earthworm meal is analyzed in terms of nutritional value, it contains high levels of amino acid, fat and minerals required for broiler feeding (10). It can be used as protein source in broiler feed, especially considering protein content and protein quality. Ghatnekar et al. (11) reported that the dry matter of earthworm contained 60-70% protein, 7-10% fat, 8-20% carbohydrate, 2-3% mineral and various vitamins. Sun et al. (12) determined that the protein content of earthworm was 54.7-71%. Bernard et al. (13) showed that the mineral content of the earthworm was 1.7% calcium, 0.90% phosphorous, 0.14% magnesium, 0.02% sodium and 0.06% potassium. The variation of nutrient composition may be attributed to different earthworm species and the different environments used to grow worms. The crude nutrient values of red worm (*Eisenia fetida*) were showed Table 1.

Earthworm protein is high in essential amino acids compared to other common feeds (12*).* It has been also reported by Dedeke et al. (10) that earthworms have an amino acid composition which is very similar to that of fishmeal and potentially superior to meat meal and contains essential amino acids as phenylalanine, leucine, lysine, methionine and valine. Red worm (*Eisenia fetida*) contains 20 out of 24 major amino acids, including the ten essential amino acids and is rich in lysine which is limiting in many feed stuff and methionine and isoleucine (16). Amino acid composition of red worm *(Eisenia fetida)* meal, fish meal and poultry meal showed Table 2.

Table 1.The crude nutrient values of red worm (*Eisenia fetida*) meal

Nutrient	Fadaee (14)	Bahadori et al. (7)	Sun and Jiang (15)	Gunya et al. (8)
Crude protein (%)	59.0	65.68	54.6	51.62
Crude lipid (%)	11.0	7.03	7.34	8.21
Crude fibre (%)	*	*	*	3.80
Ash (%)	17.0	*	21.2	19.74
Metabolic energy (kcal/kg)	*	3258	2990	*

*: Value not determined.

Earthworm protein is high in essential amino acids compared to other common feeds (12*).* It has been also reported by Dedeke et al. (10) that earthworms have an amino acid composition which is very similar to that of fishmeal and potentially superior to meat meal and contains essential amino acids as phenylalanine, leucine, lysine, methionine and valine. Red worm (*Eisenia fetida*) contains 20 out of 24 major amino acids, including the ten essential amino

acids and is rich in lysine which is limiting in many feed stuff and methionine and isoleucine (16). Amino acid composition of red worm *(Eisenia fetida)* meal, fish meal and poultry meal showed Table 2.

A number of mono and polyunsaturated fatty acids are in fairly high concentration in earthworm meal. These fats are of special interest of nutrition benefits to poultry feed (14). Essential elements such as copper (Cu), manganese (Mn) and zinc (Zn) are about one to six times higher in earthworm meal than in soybean and fishmeal (17). Earthworm body is rich in vitamin A and vitamin B compounds. It contains 0.25 mg vitamin B_1 and 2.3 mg vitamin B_2 per 100 g (12). Some mineral and fatty acid contents of red worm (*Eisenia fetida*) meal and, vitamins in body fluids of red worm (*Eisenia fetida*) were given Table 3.

Sharma et al. (18) defended that heavy metals and other pollutants could be received by earthworms and they might be transferred to the poultry that consumed these worms. Son (19) reported that there were Cd (1.23 ppm), As (4.41 ppm), Cr (1.18 ppm), Hg (0.00 ppm) and Pb (3.39 ppm) heavy metals in earthworm meal. However, Son (19) also reported that these metals were not transferred to the meat or egg, and did not affect the quality. Medina et al. (20) studied proteins from red worm (*Eisenia fetida*) in order to determine heavy metal levels, electrophoretic profile in SDS-PAGE and 2D gels, induction of humoral immune response in mice and toxicity in a human cell line. Levels of heavy metals were low and similar to tunny fish (2.5 ppm and 0.08 ppm for Pb and Hg, respectively). Consequently, they reported that *Eisenia fetida* proteins are safe for feeding animals intended for human consumption.

Table 2. Amino acid composition of red worm *(Eisenia fetida)* meal, fish meal and poultry meal

Amino acid	Red worm *(Eisenia fetida)* meal				Fish meal	Poultry meal
	Reinecke et al. (5)	Sun et al. (12)	Bahadori et al. (7)	Sun and Jiang (15)	NRC (21)	NRC (21)
Threonine	4.47	3.40	2.99	2.72	2.82	2.17
Serine	4.44	*	2.94	2.71	2.51	2.71
Valine	6.00	2.89	3.22	2.39	3.46	2.87
Methionine	1.80	1.13	1.20	1.01	1.95	0.99
Isoleucine	4.60	*	2.95	2.40	3.06	2.16
Leucine	9.80	*	5.02	3.94	4.98	3.99
Tyrosine	3.50	*	*	1.73	2.22	1.68
Phenylalanine	3.58	2.38	2.72	2.12	2.75	2.29
Histidine	3.37	1.56	1.74	1.36	1.59	1.07
Lysine	7.76	4.17	4.44	4.26	5.07	3.10
Arginine	9.56	4.07	4.41	3.27	3.81	3.94
Proline	*	*	2.41	*	*	*
Glycine	*	*	3.46	3.12	3.68	6.17
Cysteine	*	*	0.95	0.42	0.65	0.98
Tryptophan	*	*	*	*	0.78	0.37

*: Value not determined.

Pharmaceutical and other composition of red worm (*Eisenia fetida*)

Earthworms contain lumbrofebrine, terrestrolumbrlysin, lumbritin, hypoxanthine and other purines, pyrimidines, choine and guanidine. Some active enzymes occur in the yellow chloragenous cells and organs of earthworms in high concentrations, including catalase, protease, peroxidase, dismutase, β-D-glucosyl enzyme, alkaline phosphatase, esterase, S-amino-γ-ketoglutaric dehydrogenase and porphyrin synthetase. Scientists found and separated enzymes from the earthworm gut and body fluids, which can dissolve fibrin. These enzymes have been developed as innovative medicines to treat cerebral thromboses and myocardial infarction (15).

Table 3. Some mineral and fatty acid contents of red worm (*Eisenia fetida*) meal and, vitamins in body fluids of red worm (*Eisenia fetida*)

Fatty acids (3)	**%**	**Minerals(3) and Vitamins (12)**	
Lauric (C12:0)	8.4	Calcium (%)	0.82
Myristic (C14:0)	3.8	Magnesium (%)	0.10
Pentadecylic (C15:0)	1.2	Potassium (%)	0.90
Palmitic (C16:0)	6.7	Phosphorous (%)	0.90
Margaric (C17:0)	4.6	Zinc (mg/kg)	150.7
Stearic (C18:0)	17.9	Copper (mg/kg)	22.3
Oleic (C18:1c9)	4.7	Manganese (mg/kg)	26.0
Vaccenic (18:1c-7)	14.5	Iron (mg/kg)	495.3
Linoleic (C18:2c9,12) (n-6)	10.2		
Eicosadienoic (C20:2c11,14) (n-6)	2.6		
Docosanoic C22:0	1.3	A (mg/l)	13.46
Eicosadienoic C20:2c11,14 (n-6)	1.6	B_1 (mg/l)	54.65
Eicosatetraenoic (C20:4c5, 8, 11, 14) (n-6)	9.3	B_2 (mg/l)	83.06
Eicosapentaenoic (C20:5c5,8,11,14,17) (n-3)	8.8	E (mg/l)	31.64
Saturated fatty acids	45.8	C (mg/l)	292.0
Monounsaturated fatty acids	22.2		
Polyunsaturated fatty acids	31.9		

Wang et al. (22) found a kind of acid antibacterial peptide, tetra decapeptide, from red worm (*Eisenia fetida*) which has produced a disease-resistant, nutrient earthworm preparation that can be used in plant and animal production. Six antimicrobial peptides were isolated and purified from red worm tissue liquid homogenate and coelomic fluid, which contained 5–50 amino acid residues with the same or similar sequence of Ala-Met-Val-Ser-Gly, and named the antibacterial Verm peptides family according to their structure and antibacterial characteristics. Popovic et al. (23) reported that red worm (*Eisenia fetida*) has glycolipoprotein (G-90), a mixture of homogeneous tissue containing antibacterial activity against *Staphylococcus sp*. higher than commercial antibiotics such as gentamicin and enrofloxacin-5. Red worm also has lumbricin I which contain antimicrobial activity. The anti-inflammatory activity together with antioxidant properties seems to be due to the high polyphenolic content in earthworm tissues (6).

Usability of Red Worm (*Eisenia fetida*) Meal in Broiler Diets

The advantage of utilizing red worm (*Eisenia fetida*) as poultry feed are that they grow very rapidly, low inputs are required and they convert organic matter diet into high-quality protein feed (3). Many researchers have found red worm having a potential to be used as an alternative animal protein (3,5-8,20), since it has high protein content and contains 20 out of 24 major amino acids (12) which are very important in poultry production.

Reineck et al. (5) evaluated protein quality of three species of earthworms *(Eisenia fetida, Eudrilus eugeniae* and *Perionyx excavatus).* No difference in protein quality was noted between three species of earthworms, as evaluated by net protein utilization and relative nutritive values in growing chickens. Similar growth responses per g nitrogen intake were obtained for the earthworm meals, a broiler starter diet and a commercial fish meal sample. The results indicated that no harmful substances which might have had a depressing effect on the utilization of amino acids, accumulated in the bodies of the earthworms. Comparing the amino acid content of the different protein sources, showed that fish meal was highly deficient in arginine. The amino acid content of E. fetida, E. eugeniae and P. excavatus were similar and as no difference in growth was observed between these protein sources, it can be concluded that these worm proteins were equal in their ability to promote growth in chicks. It was clear that earthworm meals can serve as a suitable protein source for growing chickens. Resnawatti (24) reported that inclusion of red worm (*Eisenia fetida*) meal up to 5% level improved broiler daily feed intake and had no significant effect on body weight. However, red worm meal above 5% inclusion level resulted in depressing performance of broilers.

The protein quality of red worm (*Eisenia fetida*) meal was evaluated and compared to that of soybean meal in two experiments using broiler chicks. The results of experiment 1 indicated that there were no significant differences between red worm meal and soybean meal in protein efficiency ratio and net protein ratio, but the breast muscle weight was increased in broilers fed red worm meal diets. Moreover, the chicks fed with red worm meal had a higher crude protein digestibility. In experiment 2, feed efficiency ratio was decreased in broilers fed diets supplemented with red worm meal (3 levels of red worm meal; 0, 3 and 6% for starter and 0, 2 and 4% for grower diets). Except for breast muscle weight, the other carcass traits were not influenced by adding red worm meal in broiler diets. The breast muscle weight increased with highest inclusion rate of red worm meal. The crypt depth of jejunum was higher in broilers fed with maximum inclusion rate of red worm meal. The blood biochemical parameters of broilers were not affected by treatments. In regards with these results, it was suggested that use of red worm meal in broiler diets can be suitable (6).

Gunya (3) added to broiler diets red worm (*Eisenia fetida*) meal in different levels (0%, 1%, 3%, 5% and 10%). No significance differences were observed on average body weight, daily weight gain, daily feed intake and feed conversion ratio among different levels of red worm meal at the whole trial (1-35d). However, the highest weight daily gain was recorded in 5% and the least values were seen in 10% at 22-28d and 29-35d. The inclusion of 3% of red worm meal provided superior results in terms of carcass dressing percentage and carcass yield as compared to the control group. Red worm meal inclusion had a positive effect on intestine weight; the high intestinal weights were exhibited in 1 to 3% levels of red worm meal. The breaking strength of tibia improved with the increase of inclusion level of red worm meal in the diet. 3% inclusion level of red worm beneficially influenced the physic-chemical attributes and carcass characteristics of breast meat. In regards with these results, it can be concluded that, earthworm meal can replace fish meal at 3 to 5 % inclusion level. In another report by Gunya et al. (8); it was revealed that chicken aroma and juiciness of meat improved linearly with the increasing inclusion levels of red worm meal (0%, 1%, 3%, 5% and 10%). Nevertheless, chicken flavor scores reduced with the increasing inclusion levels of earthworm meal. In conclusion, the red worm meal inclusion levels positively influenced sensory scores of broiler breast meat, where 10% showed the best influence as compared to the other dietary treatment groups.

Bahadori et al. (7) evaluated the effects of different levels of red worm (*Eisenia fetida*) and vermihumus meal (control, 1% vermihumus meal, 1% earthworm meal+1% vermihumus meal, 2% earthworm meal+1% vermihumus meal and 3% earthworm meal+1% vermihumus meal) on broiler chicken efficiency and carcass components. The results showed that feed intake and feed conversion ratio significantly decreased with increasing the amount of red worm meal. The effect of experimental treatments was not significant on weight gain and some carcass components. The serum total protein, albumin, Ca, and P concentrations were lower in broiler chickens fed the control diet, and those variables increased linearly as dietary red worm meal increased. In like manner, humoral immune response (except heterophil/lymphocyte ratio) and relative weights of immune organs were lower in broiler chickens fed the control diet. Inclusion of various amounts of red worm meal had no effect on internal organs weights and the morphology of the ileum. Varied concentrations of red worm meal showed increased total counts of lactic acid bacteria and reduced population of pathogenic intestinal microbiota. Similarly, the meat quality of broiler chicken was markedly affected linearly by the supplementation of increased dietary red worm meal. Briefly, diets containing 3% of red worm meal can positively affect the growth performance of broiler chickens and produce meat with better characteristics.

Conclusion

The red worm (*Eisenia fetida*) has high-quality protein content, ideal amino acid composition and fatty acid profile, high protein digestibility, and antimicrobial and antioxidant properties. Moreover, it has been reported that it has no anti-nutrient factor which declines performance of broilers. Previous some studies showed that the inclusion of red worm (*Eisenia fetida*) at 3 to 5 % level to broiler diets positively affected performance parameters, carcass characteristics, meat quality and sensory scores of meat of broilers. However, red worm meal above 5% inclusion level resulted in depressing performance of broilers in some studies. In conclusion, red worm (*Eisenia fetida*) seems to be suitable using as an alternative protein source in broiler feeds.

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O[12] Effects of Replacing Soybean with Insect Larvae (Black Soldier Fly, *Hermetia Illucens* L. (1758)) in Broiler Feeding Programs

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Introduction

The worldwide increase in broiler production has a considerable environmental and economic impact, raising the urgency to find alternative feed ingredients that are more sustainable [1]. Recent studies indicate that insect might have an important function in protein and fat supply with some species having great production potential, which is the case of Black Soldier fly (*Hermetia illucens* L. (1758)) [2,3,4,5]. Due to their lack of thermoregulation, insects are more efficient converting feed and have a diminished life cycle when compared with regular livestock[6]. There is a great variety of insects, some of which are able to consume large amounts of organic matter, up to 50% of vegetable and animal waste, and promote the recycling of vital nutrients such as nitrogen and phosphorus back to the food chain[7,8,9]. The goal of the current study was to evaluate the influence of replacing soy (soybean meal and soybean oil) with insect meal (Black Soldier Fly- BSF) in broiler feeding programs.

Key words: Black Soldier fly, broilers, alternative feed ingredient, growth performance, feeding sustainability

Materials and Methods

In this experiment, 48 1-day-old Ross 308 broiler chicks were assigned to 48 individual cages. Four treatments where formed and had consisted of a basal diet where soybean meal and soybean oil were replaced by 0% (BSF0), 25% (BSF25), 50% (BSF50) and 75% (BSF75) of dry BSF larvae. The raw materials were supplied by the experimental feed factory of Estação Zootécnica Nacional, Vale de Santarém, PORTUGAL and all feeds were manufactured, with a mill and a mixer. The animals were slaughtered at 28 days of age. Body weight, daily growth and feed conversion were evaluated at a week basis, while feed and water consumption and excreta production were determined at a daily basis. Excreta characteristics and meat quality and appearance were determined at the end of the study. Dry matter (DM), crude protein (CP), crude fat (CF), ash and minerals analysis followed the Portuguese Norm 3256.

Results and Discussion

We found significant differences in live weight, average gain weight and feed conversion ratios (FCR) throughout the trial, mainly at week 2 and 3, where BSF75 and BSF50, respectively, had a significantly higher FCR ($p<0.05$) than some treatments (and always higher than control). However, when considering the trial to its full extent, these variables are not significantly different between the four treatments (Figure 1 and 2).

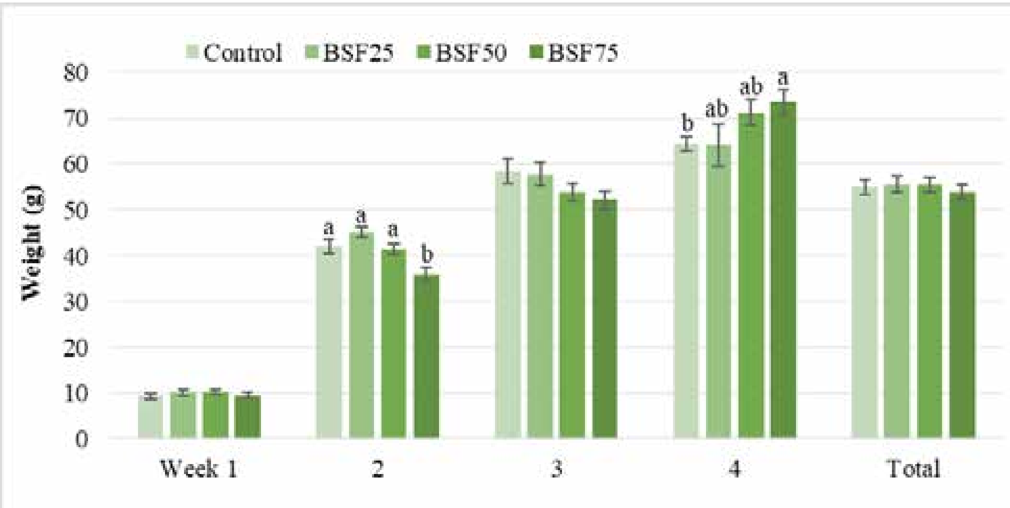
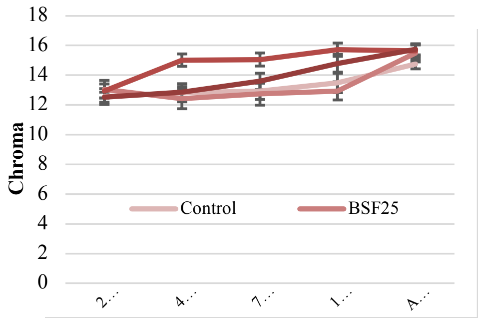
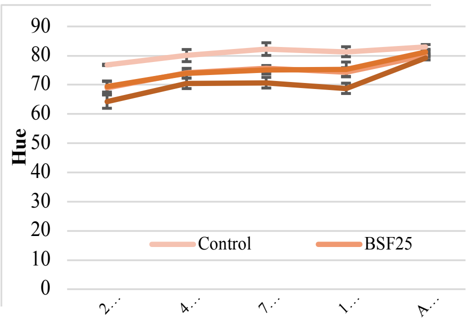


Figure 1 - Graphic representations of average gain weight through the experimental trial and in total. Different letters display significant differences (p-value < 0.05).

Figure 2 - Graphic representation of Feed conversion ratio through the experimental trial and in total.

Figure 3 - Representation of meat color changes through mean Chroma and Hue results (left and right, respectively) during the storage period (144 hours).





We also found significant differences in feed and water consumption, excreta production and meat quality criteria. In meat color measurements, BSF50 and BSF0 had higher values for Chroma and Hue, respectively, than the other treatments ($p<0.05$ and $p\leq0.005$, respectively) (Figure 3). These results associated with slightly higher than normal

pH (between 6.10-6.28) suggest that all treatments might have a tendency to be DFD (dark firm and dry) rather than PSE (pail, soft and exudative) which is the usual propensity in broiler meat production[10]. As this pH level tendency is similar in all treatments and nutritional and drip loss mean values are within normal intervals, we believe that these results were not directly influenced by the replacement of BSF larvae but for other potential influencers as age, breed or meat processing[11]. Also, when relating the size of the meat pieces with carcass weight, the only significant difference was in the breasts size, between BSF25 and BSF75, where the first is heavier.

The results of excreta analysis show multiple differences, with BSF0 having significantly lower values in most results. Besides that, animals fed with BSF larvae produced less amount of excreta (in g), and, in samples from each week of the trial, DM values were constantly higher in BSF75 treatment than others. Although this result may not appear to have any influence in the bird's wellbeing, it shows in much dryer excreta which promotes dryer litter and fewer propensities to feet problems and diseases. Additionally, the amount of calcium present in the excreta of BSF fed birds was much higher than in the control treatment. This confirms that BSF larvae are great providers of calcium and its supplementation might have enormous impact on layers nutrition and health[12].

The results also demonstrated deviations in organ size in liver relative weight and intestines relative length, where the liver from BSF0 was significantly heavier ($p=0.004$) and the intestine of BSF25 significantly shorter ($p=0.034$). Moreover, while doing the measurements of the organs we noticed that the color of cecum was strongly influenced with BSF larvae, acquiring a darker color with the increase of larvae inclusion. We believe these changes in cecum appearance were a consequence of cecum's microbiota fluctuation and different chain reactions to the supply of nutrients of animal origin, particularly chitin, a structural polysaccharide present in larvae exoskeleton[13].

Conclusion

The results of the current study demonstrate that the inclusion of dry BSF larvae does not significantly affect broiler productive performance, and therefore it may be possible to use this new alternative feed ingredient as a more sustainable nutritional solution. Moreover, there's a lot that can be improved in larvae production and processing to improve their nutritional status and better serve the broilers, or other species, nutritional needs and requirements.

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O13 DP-3Ø5423-1 (305423) Soybean in Broiler Feeding: Performance, Intestinal Morphology and Fate of Recombinant Plant DNA in the Digestive Tract

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Several genetically modified (GM) plants have been produced and approved by regulatory agencies worldwide for cultivation and commercialization. Resistance to insects and tolerance to herbicides are the most recurrent agronomic traits modified in GM crops approved for feeding livestock and poultry. Soybean plants containing the event DP-3Ø5423-1 (305423) were generated by introducing the *gm-fad2-1* gene fragment and the *gm-hra* gene. The endogenous fatty acid desaturase-2 enzyme (FAD2-1) is highly expressed in the developing soybean seed especially during the period of oil deposition, and is responsible for the conversion of oleic acid to linoleic acid. Transcription of the *gm-fad2-1* gene fragment suppresses endogenous FAD2-1 resulting in higher levels of oleic acid and reduced levels of linoleic acid, linolenic acid, and to a lesser extent palmitic acid in the seed. This study evaluated the effect of transgenic full fat soybean meal (FFSBM) and near isoline non-transgenic control FFSBM on performance and intestinal morphology of broiler chickens. In addition, the fate of transgenic *gm-fad2-1* gene fragment was examined in contents of the digestive tract. A total of 480 Ross 308 birds were randomly allocated to 24 floor pens in a 2 × 2 factorial arrangement that included diet (conventional non-transgenic and transgenic FFSBM) and gender (male and female). The starter, grower, and finisher diets were based on maize-soybean meal and provided to the birds from 0 to 10, 11 to 24, and 25-35 d of age, respectively. Birds were fed isocaloric and isonitrogenous diets containing 20% of either DP305423 or control FFSBM for 35 days. All chicks were weighed on per pen basis and feed intake (FI) was recorded at weekly intervals. Body weight gain (BWG), FI, and feed conversion ratio (FCR) were subsequently calculated to evaluate growth performance. On d 35, two birds from each replicate were selected based on average BW of each pen. To avoid any possible cross-contamination, birds fed non-transgenic FFSBM diet were sampled first, followed by birds fed diets with transgenic FFSBM. Approximately 1-cm long mucosal segments of the jejunum and ileum were excised and fixed in 10% formalin for histomorphological analysis. In addition, digesta samples were collected from the gizzard, duodenum, jejunum, ileum, and cecum for PCR analysis whereby a soybean specific *lectin* gene (189 bp) was used to identify soybean DNA. The gene *gm-fad2-1* (411 bp) inserted into DP305423 soybean was used to monitor transgenic DNA. The chicken endogenous gene ovalbumin (570 bp) was used as a positive control to test suitability of the extracted DNA samples for PCR analysis. Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (Pro13). No significant interaction was observed between treatment groups in terms of performance. As expected, male birds had higher performance compared to females during the grower and finisher phases. Dietary addition of soybean meals had no significant effect on morphological measurements of the jejunum and ileum in terms of villus height and width, crypt depth, and villus height to crypt depth ratios. However, jejunum villus height was higher in male birds compared to females. Detection of transgenic soybean DNA by PCR progressively

diminished through the GI tract being highest in gizzard content, then decreasing throughout the small intestine until reaching non-detectable levels in the cecal digesta. In conclusion, the findings of this study clearly indicate that the examined transgenic FFSBM yields similar bird performance as conventional FFSBM without any negative impact on bird health, while the recombinant plant DNA fragment degrades throughout the digestive tract.

Keywords: broiler, DP305423, transgenic soybean, performance

IS08 Veterinary Medical Products Usage Trends in Turkey

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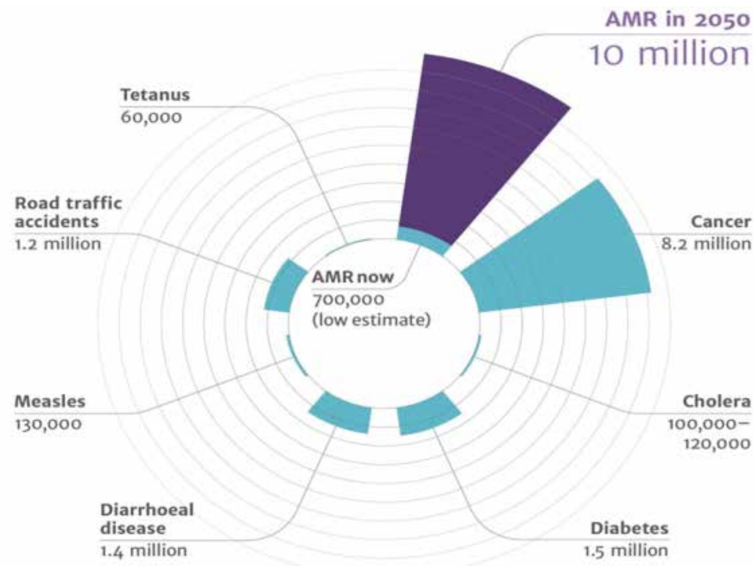
Poultry Meat Producers and Breeders Association, Ankara, Turkey

Why is Antimicrobial Resistance Important?

Antimicrobial resistance (AMR) is one of the significant issues that dwelled on both in the world and in our country. It becomes more important everyday due to the reasons of economic costs, effect on the animal health and especially on the public health.

World Health Organization (WHO) assumed the number of people who shall decease in 2018 due to cancer as 9.600.000. However, as a result of the developments related to the struggle against cancer in every year this number is expected to decrease.

Actually, there is a more critical and significant danger than cancer. According to the WHO data, if we don't struggle against AMR 10 million might die due to resistance development.



What was Done Related to AMR Until Now?

First, Northern Europe countries decided to stop using growth factor antibiotics (AGP) in 1996. In 1999, the usage of a group of antibiotics that the other EU countries were also accepted was prohibited in feed. In 2006 all growing intentional antibiotics used in feed were prohibited. In

2016 during the G20 chairmanship of China, antimicrobial resistance is the only health issue declared in the 2016 Leader's Declaration, whereas it has been mentioned also in the G20 Hamburg Leader's Declaration. Today many countries keep registers related to antimicrobial usage and track these records. In many countries unprescribed antibiotics sale is forbidden. Antibiotics prescription without antibiogram test is not accepted. Rules are set up for the usage of antibiotics with narrow spectrum and low active substances.

Situation in Turkey?

In our country the first group of antibiotics used with feed was prohibited in 1999 meanwhile with EU. In 2006, simultaneously with EU the usage of antibiotics that are used as AGP via feed was also prohibited. National residual monitoring programs were applied. In early 2006, representatives of non-governmental organizations (BESD-BİR, YUM-BİR) and Ministry of Agriculture and Forestry took a decision together related to reduce the antimicrobials used for treatment. Ministry of Agriculture and Forestry started to work on to establish a medicine tracking system. Until the activation of this system, BESD-BİR has established a Veterinary Medical product Commission to track the therapeutic purpose antibiotics by the member companies. Commission has conducted a meeting once in 2 months and took decisions for the actions to follow up the issue. Additional articles were adapted to the contracts of the contracted breeders to regulate medicine usage. One of the most significant actions was a specific software that has been designed to analyze the data within the body of BESD-BIR. Our reference for this action was the data gathering system that was used by the North Europe countries. What did we achieve by this software? Primarily data security was established and tracking of usage trends based only on active substances became available. Member companies can login the system by their own username and passwords and process data and all the companies could see the general usage without having detailed information as the name of the other companies. Especially 2016 was a year of experience and in 2017 and 2018 system became perfectly effective. All information is arranged to show the used active substance in mg/kg live weight. Especially all members agree related to the tracking of usage without the knowledge of the companies. The most significant step that will support all these was took by the Ministry of Agriculture and Forestry and in 2018 e-receipt system became active. By this system the control of the chain starting from the production of medicine till the last consumer became available.

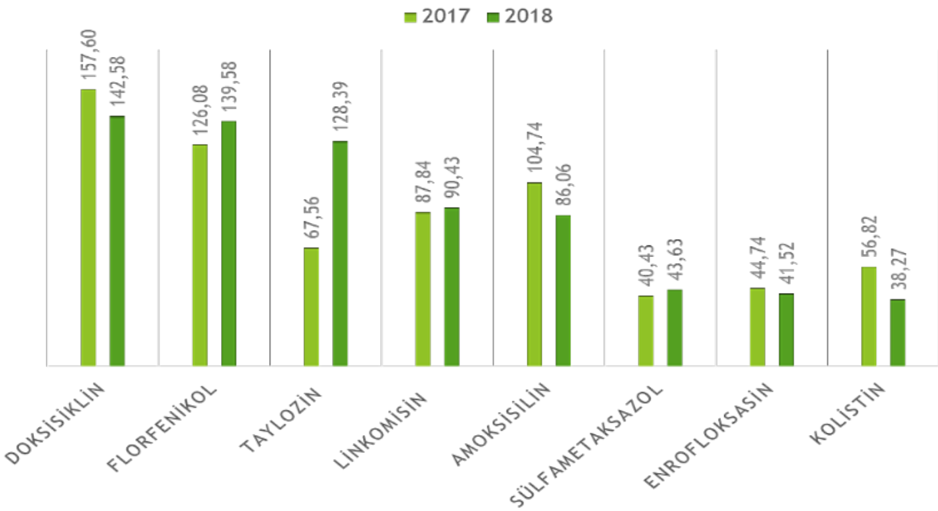
Antibiotics Usage with Figures

According to the data obtained from the software used by BESD-BIR members antibiotics usage in 2016 in poultry meat was 107,73 mg/kg. In 2017 it decreased by 35 % and recorded as 69,68 mg/kg. In 2018, a minor increase was realized and became 71,54 kg/mg. Target of the BESD-BIR with its members in 2019 is 50 mg/kg.

Table 1. Antibiotic usage in poultry production by years

Year	Total Usage (mg/kg)	Improvement compared to previous year,%
2016	107,73	
2017	69,68	35,3
2018	71,54	2,7
2019	50,00	30,1

Usage based on active substance is shown in Graph 2. Most frequent used active substances in 2017 and 2018 were doxycycline and florfenicol.



Graph 2. Quantity of used active substance mg/kg

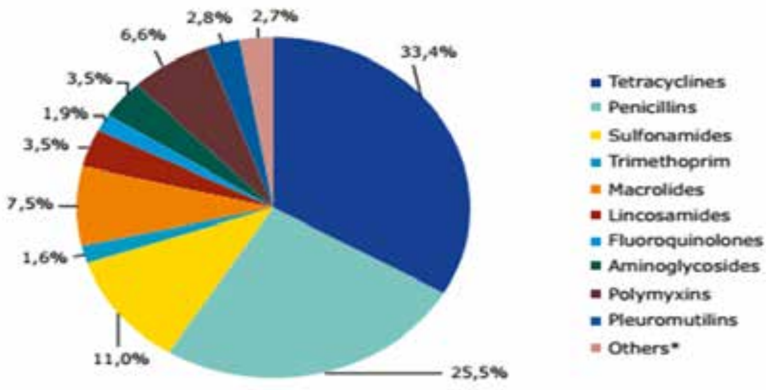
While we continue to study our production also continue to increase as poultry meat sector. In the great scheme of things, on one side poultry meat production increased and on the other side antibiotics usage has decreased. That is to say both successes have been achieved at the same time.

Table 1. Poultry meat production in Turkey

	Poultry Meat (tons)	Turkey Meat (tons)	Total (tons)	Increase compared to previous year, %
2016	1 879 018	46 501	1 925 518	
2017	2 136 734	52 363	2 189 097	13,68
2018	2 156 671	69 536	2 226 207	1,69

Source: TÜİK

When we check the numbers related to EU, according to ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) data the most used antibiotics group is tetracycline. Penicillin group antibiotics are the second most used antibiotics group after tetracycline group.



Graph 3. Antibiotics usage in European Union Countries (ESVAC 2013)

Major countries in antibiotics usage are South Cyprus, Spain, Italy, Portugal and Germany. When compared with the EU countries it could be seen that antibiotics usage in our country is lower than the EU average.

Table 2. Antibiotics usage in European Union Countries (ESVAC 2013)

Country	Sales(tonnes) for food-producing animals	PCU(1.000 tonnes)	mg/PCU
Austria	54.7	957	57.2
Belgium	259.5	1,657.5	156.6
Bulgaria	46.5	400.9	116.1
Cyprus	47.9	112.5	425.8
Czech Republic	57.2	696.8	82.1
Denmark	108.5	2,418.4	44.9
Estonia	8.5	137.2	62.2
Finland	12.5	514.4	24.3
France	681.0	7,165.4	95.0
Germany	1,527.2	8,525.6	179.1
Hungary	175.7	763.1	230.2
Iceland	0.6	115.2	5.3
Ireland	99.6	1,761.6	56.5
Italy	1,318.4	4,371.9	301.6
Latvia	6.2	167.1	37.0
Lithuania	12.4	339.5	36.6
Luxemburg	2.7	51.0	53.6
Netherlands	225.6	3226.3	69.9
Norway	6.6	1,788.6	3.7
Poland	575.6	3,806.2	151.2
Portugal	179.4	958.2	187.2
Slovakia	15.5	247.8	62.5
Slovenia	4.0	180.2	22.4
Spain	2,201.9	6,943.6	317.1
Sweden	10.0	795.6	12.6
United Kingdom	422.0	6,799.1	62.1

What is the Target?

Descriptions in data collection system are completed and updates will permanently continue. Antibiotics having critic importance for human health will be followed closely and usage in poultry shall be minimized. For this reason, the active substances that are used for poultry are regulated as indicated in table 3 based on OIE list. This list will be updated regularly, and a road map related to active substance usage according to its importance level shall be determined. The system will give a warning for the BESD-BIR members that input data in this matter if the usage of active substance included in the class that has the critical importance for human health increases. We will continue to work to decrease 2019 average to 50 mg/kg. RESPONSIBLE ANTIBIOTICS USAGE principles will be followed during treatment.

Table 3. Classification of antimicrobials used for poultry diseases according to their importance level

Classification of antimicrobials used for poultry diseases according to their importance level

Importance level	Groups	Active substance
Critically important antimicrobials	Aminoglycosides	Spectinomycin, neomycin
	Amphenicol	Florfenicol
	Cephalosporin (3rd generation)	Ceftiofur, ceftriaxone (no usage for poultry)
	Macrolide	Erythromycin, tilmicosin, tylocin, tyvalosin
	Penicillin	Amoxicillin
	Quinolone (2nd generation)	Danofloxacin, enrofloxacin, sarafloxacin
	Sulfonamide	Sulfaklorpridazin, sulfadiazine, sulfadimethoksine, sulfadimethosazol, trimethoprim, sülfaklozin
	Tetracycline	Chlortetracycline, doxycycline, oxytetracycline
	Polypeptide	Colistin
High importance antimicrobials	Lincosamide	Lincomycin
	Kinnelon	Flumequine
Important antimicrobials		Toltrazuril
		Amprolium

Source :BESD-BİR

O[14] Determination of *Listeria monocytogenes* and Antibiotic Resistance Profiles in Organic Poultry Meat

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Abstract

In this survey from October to December 2018, 240 samples of packaged organic chicken products (n=80 wings, n=80 whole chicken leg, n=80 without skin-breast) were randomly collected from the province of Samsun in order to investigate the presence of *Listeria monocytogenes*, In this context; i) *L. monocytogenes*,. in the samples were isolated using IMS method with conventional cultivation techniques ii) Confirmation of presumptive isolates were completed using PCR, iii) Antimicrobial resistant profile were determined of isolates. As a result of the analysis; contamination with *L. monocytogenes* were detected in 60 (25%) of 240 chicken samples. Research findings based on sample distribution shows that: 24 (24/80-30%) of whole chicken leg samples, 20 (20/80-25%) of wing samples and 16 (16/80-20%) of without skin-breast samples were contaminated with *L. monocytogenes*. Twenty six of the isolates (27%) were resistant to Ampicillin. Twenty three (23.9%), 14 (14.5%), 13 (13.5%), 12 (12.5%), 9 (9.3%), 7 (7.2%), 5 (5.2%), 4 (4.1%) and 3 (3.1%) of the isolates were resistant to meropenem, tetracycline, sulfamethoxazole/trimethoprim, penicillin G, amoxicillin/clavulanic acid, vancomycin, oxytetracycline, erythromycin and chloramphenicol respectively. Multidrug resistance properties were observed 12 *L. monocytogenes* isolates.

O15 Virulent Effects of a Bacteriophge Against Salmonella Enteritidis MET-S1-411 at Different Storage Temperatures

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Abstract

The food industry is one of the most important industries in the world in terms of supplying the basic nutritional needs of people. Microbial contamination in relation to foodborne diseases and food spoilage is an important problem for food industry and food safety. The use of bacteriophages as the biocontrol agent in the prevention of this problem is one of the most effective methods. Bacteriophages shows specific antimicrobial effect to the target host in addition to being harmless to humans, animals and plants. The aim of this study was to determine the virulent effects of a bacteriophage (SEnt-F1) against *Salmonella* Enteritidis MET-S1-411 at different storage temperatures (4 and 25 ° C). Chicken breast samples were contaminated with 10^3 and 10^6 cfu/g *S*. Enteritidis and treated with bacteriophage at 10^9 pob/g. In chicken meat with contaminated at 10^3 cfu/g, the count of *S*. Enteritidis was below the detectable level at 4 and 25°C. In addition, the infectious effect of bacteriophage in chicken meat contaminated at 10^6 cfu/g was found to be higher at 25°C.

O[16] Phylogenetic Analysis of Salmonella Enteritidis Strains Isolated from Broiler Breeder and Hatchery by Pulsed Field Gel Electrophoresis

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Abstract

Among *Salmonella* serotypes, *Salmonella enterica* serovar Enteritidis is the most isolated serotype in both humans and animals worldwide. It causes gastroenteritis in humans, while it is asymptomatic in animals. In this study, *Salmonella* Enteritidis strains isolated from materials from different flocks located in western Black Sea region of Turkey are examined in our department between 2014-2017 for molecular typing and phylogenetic analysis by Pulsed Field Gel Electrophoresis (PFGE). For this purpose, a total of 64 *S.* Enteritidis strains isolated from broiler breeder and hatchery and also only one *S*. Enteritidis strain isolated from a worker were examined. PFGE analysis revealed two different PFGE groups and four different subgroups. According to the similarity matrix created using the unweighted pair group method with arithmetic averages (UPGMA) with dendograms of the PFGE profile patterns of *S.* Enteritidis strains, minimum similarity was 66% and maximum similarity was 100%. It was determined that 92% of the strains were close to each other according to the Tenover, and the similarity ratio was close to 100% and had the same genotype. The high similarity rate among strains isolated from broiler breeders and hatchery from different flocks showed that there are major genotypes in this region. The isolation of strains with a single genotype from different commercials in the West Black Sea region suggests that there may be a crossing between the flocks.

IS09 Environmental Sustainability of Poultry Meat Production

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Summary

The main effects of planet human growth, urbanization and change in eating habits are discussed. Undernutrition and malnutrition are still high in many developing countries and food of animal origin can increase the nutritive value of human diet. The increasing demand worldwide for food of animal origin, particularly in developing countries, is a challenge for agriculture and the livestock sector, already facing criticism in terms of environmental sustainability.

Meat production is considered by the public opinion as one of the most responsible human activities for climate change, water contamination, acidification of the eco-systems, loss of biodiversity, etc. However, monogastrics and particularly poultry can be very efficient and sustainable, supplying high quality food which can supplement food of plant origin in a balanced human diet.

Introduction

Planet population has doubled between 1960 (3.5 billion) and 2010 and is expected to reach 9-10 billion people by 2050. At the same time, people living in urban areas were about 33% in 1960, 50% in 2010 and will be around 67% in 2050. The combined action of human growth and urbanization, particularly remarkable in the developing countries and in the recently developed countries, is a challenge in terms of food security, particularly considering that soil and water - the main limiting factors in agriculture - cannot be increased. By contrast, water is getting more and more precious and less available in many areas of the world where desertification is increasing. Climate change, due also to human activities, animal production included, makes snow and rain less regular in frequency and intensity, with a negative impact on agriculture and livestock production. At the same time, there is a growing demand for food of animal origin due to the increasing income of the population worldwide, particularly in developing countries (12).

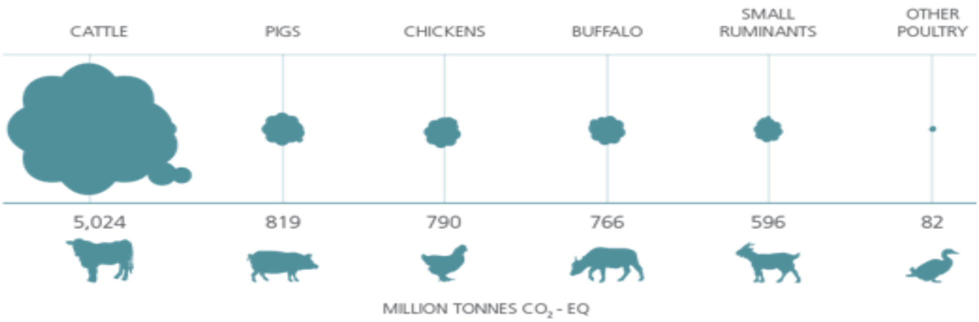
Since 1990, world's population has grown at a rate of 1.1%, children mortality within 5 years of age has dropped from 9 to 4.8%, and malnutrition of under-five children, expressed as underweight, has decreased from 24.9 to 15.1% (23). However, there is still a major disparity between low and high income countries. Particularly, in low and lower middle income countries (which account for almost half of world's population) the demographic growth is much higher (1.5-2.3%) than in richer areas, and child mortality (6.1-8.2%) and malnutrition (21.8-24.1%) still high and unacceptable. Global demand for meat and milk is expected to increase by 57% and 48%, respectively between 2005 and 2050 (1); in the same period, poultry production will increase by 90% and by 28,3 million tons (MT) in the period 2014-2023 (2).

Environmental Impact of Different Livestock Sectors

The livestock sector has a negative impact on the environment, since it: 1) contributes to the production of greenhouse gases; 2) releases nitrogen, phosphorus, heavy metals and antibiotic residues into soil, water and air; 3) uses soil, a non-renewable and limited resource, for the production of animal feeds. Animal production is responsible for 14.5% of greenhouse gas (GHG) emissions measured as CO_2 equivalents (CO_2-eq) (12).

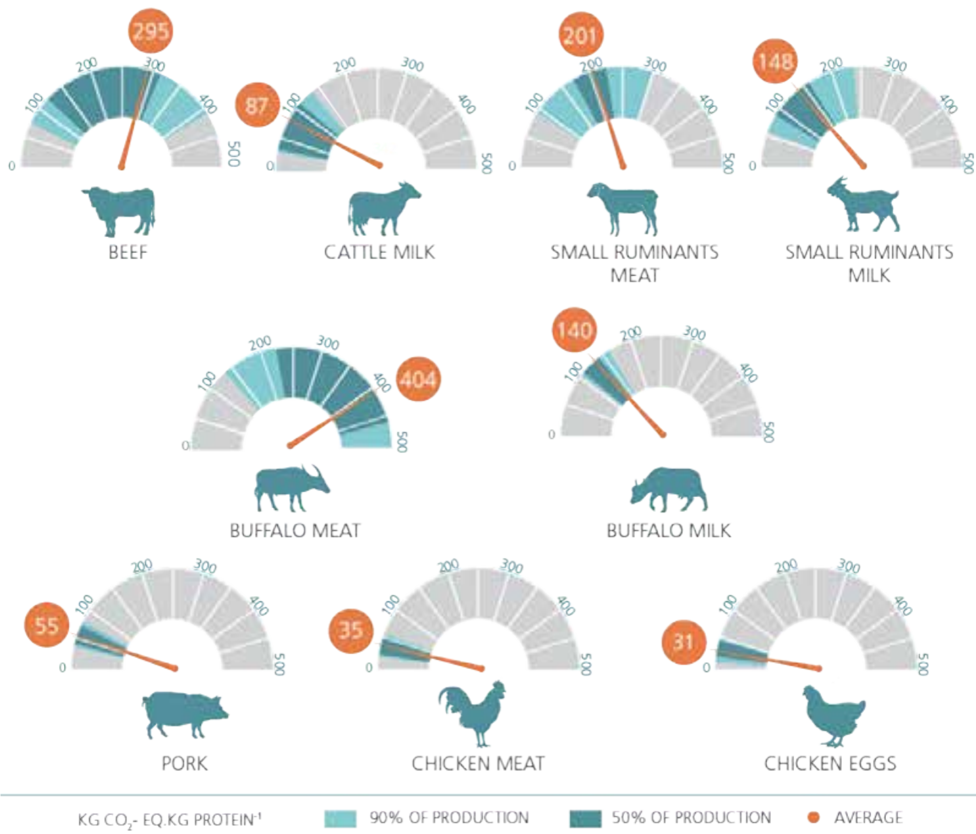
Cattle are the main contributor with about 5.0 MT CO_2-eq, (62% of total emissions); pigs, poultry, buffaloes and small ruminants have much lower emissions (fig. 1).

Figure 1 - Global estimates of emission by species (13).



Poultry meat and egg productions, in particular, contribute for just 8% to livestock sector emissions (12) and represent one of the most consumed food of animal origin in the world because there are no major taboos on their consumption and due to the low production costs. Moreover, poultry meat (chicken and turkey) productions are very efficient and environmentally sustainable in comparison with other meat products (12; 22; 25), mainly due to the high daily weight gain, the excellent feed conversion ratio (FCR), the short production cycle and the high carcass yield and meat-bone ratio.
Also when the impact is expressed as kg CO_2-eq/kg protein, the emissions derived from poultry productions result to be lower in comparison with other species. Particularly, chicken meat impacts 8.4, 5.7, 2.5 and 1.6 times less than beef, mutton, cattle milk and pork, respectively. Only eggs resulted slightly less impacting (fig. 2).

Figure 2 - Global emission intensities by commodity, expressed as kg CO_2-eq/kg protein (13).



The efficiency of utilization of the more easily digestible feed nutrients (non-structural carbohydrates, proteins, lipids) and of the energy they supply make monogastrics competitive with ruminants and preferable to them for meat production when non-forage feed are available. A hundred kg feed (composed by 80% cereal grain and 20% protein supplement) can produce about 45 kg chicken meat, 35 kg pork and only 15 kg beef (9).

Indeed, there is a close link between the environmental sustainability and the ability to convert feed into food (FCR) in different species. Particularly, animals with low feed conversion efficiencies, such as beef, showed high values of global warming potential (GWP), whereas broilers, with high feed conversion efficiencies, registered low GWP values (7; 22). Recently, Bava et al. (4) reported higher emissions and excretions per live weight in the last finishing phase of heavy pig production system due to the high increase of the FCR. Also in poultry production, Cesari et al. (7) registered higher GWP per kilogram of carcass weight in heavy broilers in comparison with light and medium weight animals.

In all species the rearing system affects the environmental impact of meat production (table 1). For example, in the intensive broiler production system, the GWP, expressed as kg CO_2-eq/

kg meat, resulted lower in comparison with free range system (4.4 *vs.* 5.1, respectively). The data reported in table 1 show that the environmental impact can be assessed also per amount of nutraceutical substances such as omega-3 fatty acids or, more particularly, the EPA and DHA, that animals are able to produce thus supplying them to humans. Also in terms of the omega-3 production, chicken resulted the less impacting animals, particularly the intensive reared ones.

Table 1 - Global warming potential (GWP) of meat production expressed per species, rearing system and different functional units (modified from McAuliffe et al., 2018) (20).

Species	System	Mass-based GWP (kg CO_2-eq/kg meat)	Quality-based GWP (kg CO_2-eq/g Ω-3)	Quality-based GWP (kg CO_2-eq/g EPA+DHA)
Beef	Concentrate	9.8	48.0	288.1
	Forage	18.3	18.5	67.7
Lamb	Lowland	26.1	28.7	99.2
	Upland	30.9	30.0	98.9
Chicken	Intensive	4.4	1.2	25.1
	Free range	5.1	2.4	34.7
Pork	Intensive	7.4	14.4	50.3

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Environmental Impact of Poultry Systems

Data reported above show that poultry production is already a very environmental friendly source of animal protein. Despite this, thanks to the future results of scientific research and the continuous progress in rearing techniques, it is possible to hypothesize a further reduction in the environmental impact of this sector.

Even if feed production, processing, and transport represent the main contributor to environmental impact of chicken meat production, the improvement of some aspects of the productive cycle, such as the reduction of slaughter weight in order to improve feed efficiency, the choice of the most suitable stocking density and the productive systems could improve the environmental sustainability of broiler production.

Effect of Stocking Density on Environmental Impact

Stocking density is considered an important parameter able to affect the environmental impact of the broiler sector (7; 10; 16).

In general, non-EU countries have limited or no legislations on animal welfare and broiler protection, whereas in Europe, important producer of poultry meat, in 2007 the Council Directive 2007/43/EC (8) (concerning the laying down minimum rules for the protection of chickens kept for meat production) was adopted. This directive fixed in the broiler farms the maximum stocking density at 33 kg live weight (LW)/m^2. When additional criteria (regarding management, monitoring, animal welfare and mortality) for the use of increased stocking density are respected, the maximum stocking density does not at any time exceed 42 kg LW/m^2.

The high stocking density applied to maximize profit per unit area, however, results in a negative poultry health and welfare, even if different studies (5; 11) reported inconsistent results about the effect of stocking density on welfare.
Recently, Cesari et al. (2017) (7) reported the effects of increasing stocking density (33 *vs*. 42 kg LW/m^2) on the environmental impact of heavy broiler meat production (table 2).

Table 2 - Variation of the environmental impacts (expressed per kg live weight, LW, at fattening farm gate) of heavy broiler production at increasing stocking density (Cesari et al., 2017, modified) (7).

Impact categories		33 kg LW/m^2	42 kg/LW/m^2
Global warming	kg CO_2-eq	3.84	4.10
Acidification	g SO_2-eq	19.2	20.2
Eutrophication	g PO_4^{3-}-eq	12.8	13.4
Terrestrial ecotoxicity	g 1.4-DCB-eq	5.00	5.27
Non-renewable fossil energy	MJ	12.4	13.0

The authors reported that animals kept at a higher stocking density (42 kg LW/m^2) impact slightly more (5% on average) in comparison with broilers reared with a stocking density of 33 kg LW/m^2. Therefore, the rise in meat production due to the increase in stocking density does not lead advantages in terms of environmental impacts, because the increase in density is associated with a worsening of the FCR and an increase in mortality.

Leinonen et al. (2014) (16) underlined small differences on the overall environmental impact between a low density system (30 kg LW/m^2) and the standard indoor one (37 kg LW/m^2). However, differently from what found by Cesari et al. (2017), the values of GWP of broilers reared at lower density is slightly higher (2%) in comparison to that of animals reared with a standard density (table 3), for the higher electricity and liquefied petroleum gas consumption. The highest contribution to GWP was given by feed production, processing and transport (2.95 and 3.08 t CO_2 eq/t expected carcass weight for broilers reared at 30 and 37 kg/m^2, respectively).

The higher consumption of liquefied petroleum gas for the increase heating requirements, moreover, increased the value of primary energy use for animals reared at 30 kg LW/m^2 in comparison with that obtained for broilers reared at higher density. Conversely, for acidification and eutrophication potentials, the results found by Leinonen et al. (2014) (16) for the low density system were lower in comparison with those of the standard system due to a worse FCR.

Table 3 - Global warming potential, primary energy use, eutrophication and acidification for the different broiler systems considered per 1,000 kg of expected carcass weight (Leinonen et al., 2014, modified) (16).

Category	30 kg LW[a]/m^2	37 kg LW[a]/m^2
Global warming potential (t of CO_2-eq)	4.42	4.35
Primary energy use (GJ)	28.0	24.9
Eutrophication (kg PO_4^{3-} eq)	19.2	20.5
Acidification (kg SO_2 eq)	43.3	47.0

[a] Live weight.

Effect of Different Productive Systems on Environmental Impact

In general, in most countries live weight of chickens at slaughter varies between 2.0 and 2.5 kilograms. By contrast, in Italy the majority of chickens slaughtered are medium and heavy broilers (3 kg LW on average).

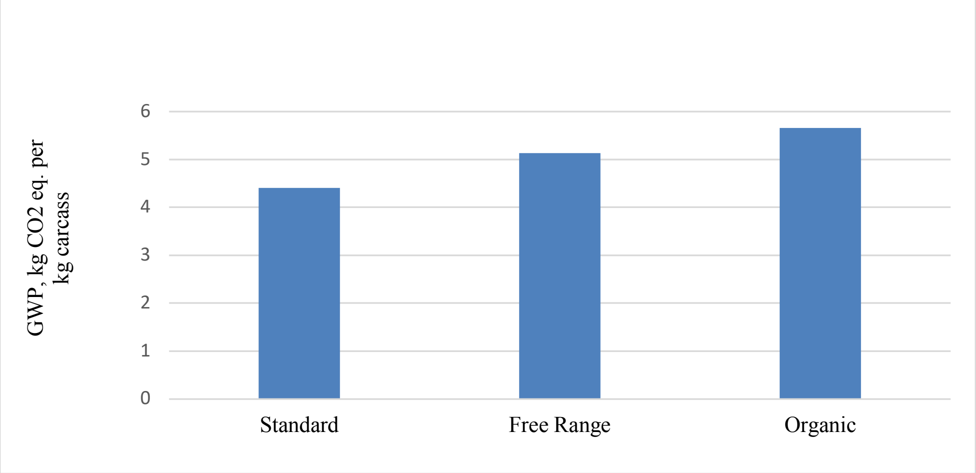
These categories of weight significantly affect the environmental impact of the broiler sector. Indeed, Cesari et al. (2017) (7) recently studied the effect of slaughter weight on environmental impacts (table 4) and underlined that heavy broilers (characterized by a LW of 3.8 kg at 53 days of age) had the higher impacts per kg LW for all the categories in comparison with light and medium animals (1.6 and 2.5 kg LW at 32 and 40 days, respectively). The highest impact of heavy broilers is mainly due to a less favorable FCR (1.88) in comparison with the other two groups of animals (1.63 and 1.50 for medium and light LW broilers, respectively) since feed conversion is correlated with the LW and the age of animals.

Table 4 - Environmental impact of broiler production, expressed per kg of live weight (LW) at fattening farm gate (Cesari et al., 2017) (7).

		Broiler production		
Impact categories		Light broilers 1.6 kg LW	Medium broilers 2.5 kg LW	Heavy broilers 3.8 kg LW
Global warming	kg CO_2-eq	3.03	3.25	3.84
Acidification	g SO_2 eq	14.3	15.8	19.2
Eutrophication	g PO_4^{3-} eq	10.0	10.6	12.8
Terrestrial ecotoxicity	g 1.4-DCB eq	4.80	4.69	5.00
Non-renewable fossil energy	MJ	10.2	10.7	12.4

Leinonen and Kyriazakis (2016) (18) reported the results (fig. 3) of a previous study in which three different productive systems (standard indoor, free-range and organic) are compared. The authors determined a lower impact in terms of GWP, primary energy use, eutrophication and acidification potentials, abiotic resource use, and land use. In particular, GWP of the standard system was lower in comparison with the free-range and organic systems (4.41 *vs*. 5.13 and 5.66 kg CO_2-eq/kg carcass weight, respectively), mainly for the higher feed consumption due to the longer production cycle in free-range and organic systems. The same results were reported by Castellini et al. (2012) (6): comparing conventional, organic, and organic-plus (characterized by the use of slow-growing strains and a wider outdoor space, 10 m^2/bird) poultry production systems, they confirmed a higher environmental impact in organic and organic-plus poultry systems.

Figure 3 - Global warming potential (GWP) for different broiler production systems (Leinonen and Kyriazakis, 2016, modified) (18).



When comparing the results of some studies (table 5), it can be noticed an increase of all the impact factors studied for the free-range production system, in comparison with the standard one. In particular, da Silva et al. (2014) (10) compared two different production systems. The first system is characterized by a density of 22 animals/m^2 and a rearing time of 40 days of age (France standard system), while the second uses slow-growth strains, a low density equal to 11 animals/m^2 and a rearing time of 89 days (French high-quality system Label Rouge). These authors reported largest impacts of all the environmental factors for the extensive broiler production system, probably for the worsening of the FCR.

Table 5 - Comparison between relevant publications, per ton of live weight at the farm gate[a].

	System[b]	C[c]	Acidif. kg SO_2-eq	Eutrophic. kg PO_4^{3-}-eq	Climate change kg CO_2-eq	Land use m^2/yr	Cumul. Energy demand GJ
da Silva et al. (2014)	ST	FR	28.7	13.8	2216	2676	19.1
da Silva et al. (2014)	LR	FR	47.2	19.3	2696	3903	29.5
Leinonen et al. (2012)	ST	UK	32.7	14.2	3087	3920	17.8
Leinonen et al. (2012)	FrR	UK	40.0	16.2	3437	4824	17.2
Williams et al. (2009)	ST	UK	25.9	14.0	1800	4270	11.2
Williams et al. (2009)	FrR	UK	30.8	23.5	2000	6700	11.2

[a] Functional unit: t LW=tonne of live weight. Williams et al. (2009) (26) and Leinonen et al. (2012) (14) used carcass weight as functional unit. We transformed carcass weight into live weight assuming a carcass yield of 70% for standard systems and 67% for free-range systems.
[b] Standard (ST), Label rouge (LR), Free range (FrR).
[c] Country: FR=France; UK= United Kingdom (10).

Feed Production and Environmental Impact

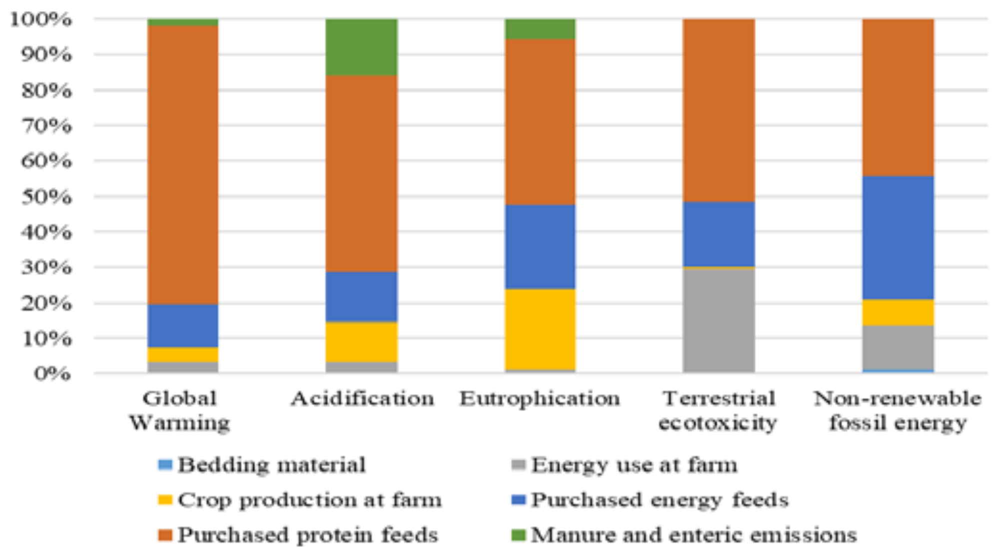
Different studies on monogastric species (4;7) indicated that feed production is the major contributor to environmental impacts.

Figure 4 shows that in poultry production purchased feeds had an important role in impacting different categories; in particular, the production of purchased feeds (both energy and protein feeds) accounted for 92% of GWP and 76% of Acidification Potential (7).

Moreover, the production of protein feeds (especially soybean meal) resulted the most impacting (from 42 to 79%) for the following categories: GWP, Acidification and Eutrophication potentials, Terrestrial ecotoxicity and Non-renewable fossil energy. The high contribution to environmental impact of soybean meal is due to land use change (LUC) because soybean production is associated with deforestation-related emissions. Also litter and manure management and enteric emissions have an important role on GWP and on other main categories of impact.

In the study of Leinonen and Kyriazakis (2016) (18) the most important contribution to GWP was represented by feed production. The other sources of impacts, in terms of importance, were represented by the electricity, gas and oil used in broiler farms and by housing and manure management.

Figure 4 - Contributions of different compartments to environmental impact categories (per kg live weight) for broiler production at fattening farm gate (Cesari et al., 2017) (7).

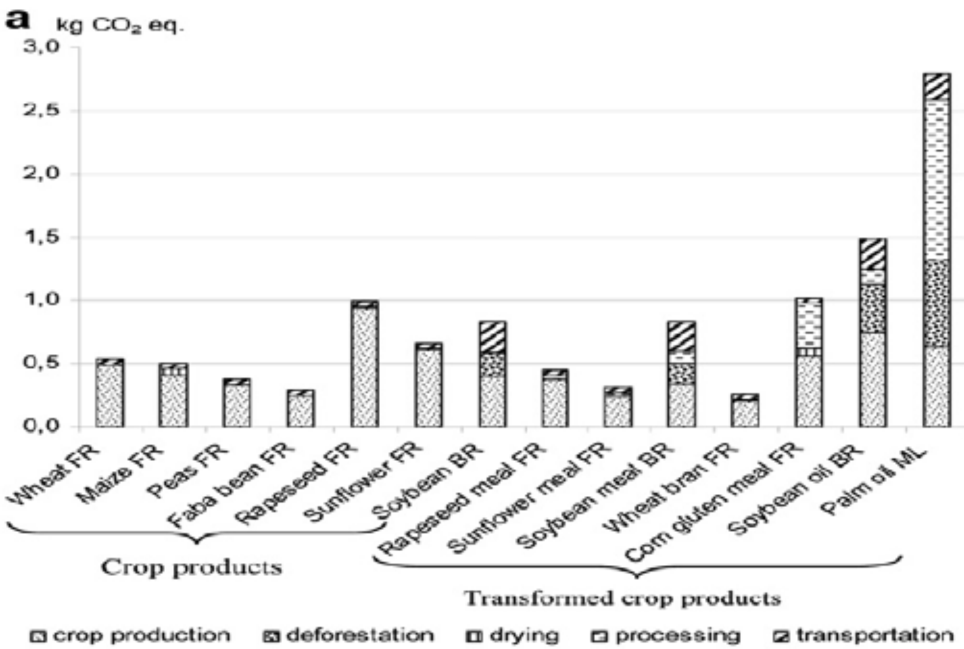


The use of soybean meal in broiler diets undoubtedly affects the environmental impact of broiler sector because this protein feed generally origins from South America and its production is related to GHG emissions often determined taking into account land use change (LUC).

Although in different regions of the world the soybean meal production is really related to this problem, some countries have developed sustainability assurance protocols that identify the best processes and management practices in order to reduce the environmental impact and to guarantee sustainable soybean production (24).

Nguyen et al. (2012) (21) reported the impact of different raw materials on GWP and underlined the higher impact of soybean meal in comparison with rapeseed meal and sunflower meal for the deforestation and transportation of soybean meal from Brazil to France. Moreover, they reported that the environmental impact of broiler was greater when energy and protein level of the diet increased.

Figure 5 - Climate change impact per kg of feed ingredients delivered at the feed mill in Bretagne (FR: France; BR: Brazil; ML: Malaysia). (Nguyen et al., 2012) (21).



The possibility to use alternative protein sources to reduce GHG emissions has been investigated by some studies (21; 15; 18; 21). In particular, Leinonen et al. (2013) (15) reported a lower value of GWP (up to 12%) when soybean meal of the diet was substituted by beans, peas or rapeseed at high level (up to 300 g/kg); when these alternative protein sources were added to the diet at lower and more sounded levels, the reduction of GWP became not statistically different. This could be explained considering that the replacement of soybean meal with high percentage of beans, peas or rapeseed leads a partial substitution of some raw materials (such a wheat) and requires the addition of other ingredients (e.g. vegetable oil) which determined a greater GWP.

On the other hand, in this kind of studies the GWP values strongly depend on the different methods used to consider LUC in the analysis.

Moreover, it is necessary to consider that the addition of pure amino acids to obtain a balanced profile of the diets formulated with alternative protein sources reduces the beneficial environmental effects related to the reduction of soybean meal for the higher emissions of GHG associated with amino acids production.

Also Baumgartner et al. (2008) (3) found that the inclusion of alternative sources in substitution of soybean meal in different animal species did not lead to an overall environmental improvement and they suggest to consider the whole diet, and not only a single raw material, in the evaluation of the benefits related to the use of alternatives protein sources.

In the studies cited above, it was assumed that broiler performance was the same when the animals were fed soybean meal or alternative protein sources, but if the FCR gets worse for the use of alternative sources, the impacts (per unit of the final product) increased.

The addition of specific enzyme (protease) to the diet, useful to improve protein utilization, could be a strategy to reduce dietary protein and, consequently, to decrease emissions. In particular, recently Leinonen and Williams (2015) (17) reported that GWP was reduced for the lower inclusion of soybean meal in the diet (for the lower carbon dioxide emissions from land use changes related to soya production), while the reduced value of eutrophication and acidification was due to the lower emission of NH_3 derived from housing and manure management.

Conclusions

Thanks to the genetic improvement that will be carried out in the coming years and that shows a scenario (19) where there will be a significant reduction in the length of the rearing cycle (from 34 to 27 days to reach live weight of 2 kg) and to an overall improvement on performance, GWP will be reduced by 9%, Eutrophication potential by 12%, Acidification potential by 10% and Abiotic resource use by 9% The continuous improvement in FCR, also obtained thanks to more efficient breeding techniques, will allow to reduce protein and energy feed destined to broilers decreasing the environmental impact of this livestock production. Moreover, also a correct manure management and the possibility in the future to use some plant oil by-products (from soy and rape seeds) and alternative protein sources (insect and invertebrate meals) in broiler diets could reduce the environmental impact of this sector.

Finally, precision farming and precision feeding will allow to improve more and more the efficiency of poultry meat production and, consequently the economic and environmental sustainability of this important livestock sector.

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IS10 Welfare and Its Relationship with Performance and Meat Quality in Broilers

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Abstract

Broiler welfare can be defined as a bird's ability to cope with the environment. Welfare assessments in broilers can be based on physical (e.g., injuries), behavioral (e.g., walking, pecking), physiological (blood biochemical parameters), and production-related (body weight, feed conversion). Broiler welfare is affected by genetic background, slaughter age/weight, house conditions, and preslaughter catching, crating and transportation. The main objectives of this review are to introduce the valid indicators of broiler welfare and to discuss the relationships among welfare, performance, and meat quality traits in broilers.

O[17] Broiler Performance of Pure Lines and Efforts for Obtaining Broiler Grandparent Stocks

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Abstract

In this paper, some results of the studies to produce broiler parents were evaluated in our country. The yield traits of the fast-feathering two males (B1 and B2) and slow-feathering two (A1 and A3) and fast-feathering (A2) pure-line females were evaluated for 3 years, the grand-parent performances produced for trial purposes and the broiler performances of pure lines were evaluated. Live weight, breast width, feed efficiency and fertility rate in male lines; live weight, feed efficiency and egg production are used as the main selection criteria in female lines.

The feed consumption and live weights of the rearing period in pure lines were similar to the effect of controlled feeding. Male lines have a higher live weight. In addition to the results of the first year, rearing period mortality rates were found acceptable (3.5% to 7% in female lines and 2.1% to 6.5% in male lines). 50% egg production age was determined between 180 days and 193 days in female lines; 181 and 197 days in male lines. At 40 weeks age, between 64 and 84 eggs in female lines; 48 and 69 eggs were produced in male lines. The age of reaching the hatching weight of the egg weight was 50% egg production age (26-28 weeks) for all pure-lines. Mortality rates were found between 0.7% and 6.7% in the females; 0.3% and 3.4% in the male lines. Fertility rates were determined between 89% and 93% in all pure lines.
Grandparent male (B1xB2) and female (A2xA1) lines produced for experimental purposes were respectively 188 and 183 days at 50% egg production age, 40 weeks egg production were 78 and 60 eggs; and 54 weeks production was determined 139 and 104 eggs. It was reached to hatching egg weight at the 50% egg production age.

The broiler performances of chickens produced from pure lines were maintained at an acceptable level for 3 years. At 6th week, male-female mixed live weights ranged from 2319 g to 2624 g in female lines and between 2663 g and 2918 g in male lines. Feed efficiencies were found between 1.65 and 1.78 in female lines; 1.67 and 1.77 in male lines.

Rearing and egg production period performance tests of 2 male and 6 female parents, which are produced by reciprocal crossing from male female lines are continued. When the broiler tests of 12 hybrids produced from these parents will be completed, the most appropriate parent and hybrid production way will be introduced from the pure lines.

Parents produced from existing pure lines were named Anadolu-T and shared with private sector with small capacities for trial purposes. Although the initial results are positive, all results have not yet emerged. It is seen that there is hope of pure lines at hand.

Keywords: Pure-lines, grandparents, broiler, performance tests

IS[11] Biosecurity and Food Safety: Concerns and Solutions in Poultry

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Abstract

Poultry production and trade as a source of valuable and cheap protein for securing nutritional demands of human population become one of the fastest growing livestock industry. However, along with increase of production advancement in genetics, production technologies and disease prevention, hazards related with production economics and risks to human health still remain major concerns to producers and consumers.

Earlier studies concluded that every poultry farm has its own risk profile for the introduction of pathogens, subsequent development of disease and spread of pathogen to other farms. Implementation and maintaining of biosecurity measures and plans can reduce but not fully eliminate the risk of pathogen introduction and their consequences. The Food and Agriculture Organization of the United Nations has defined four types production systems interalia based on biosecurity measures implemented. Nevertheless experience and studies on transmission of highly pathogenic diseases such as Highly Pathogenic Avian Influenza had not showed scientific evidence for distinguishable difference in disease containment among them. From the public health perspective, zoonotic poultry pathogens such as campylobacter and salmonella still remain major food safety concerns.

This paper provides summary of current knowledge, scientific data, and recommendations on poultry production surveillance, biosecurity, and food safety concerns. Our intention was to elaborate efficiency and effectiveness of application of scientifically documented practices and to discuss solutions to enhance both industrial and governmental capacity in prevention of animal health and welfare disturbances and its consequent impact on human health and market stability.

Introduction

The global growth of the livestock sector is expected to continue, mainly due to demand driven by increase of human population and urbanization. Poultry meat is expected to have the highest growth, with 121% (16). Most of this growth will be driven by need of Asia market. The majority (92%) of poultry meat production comes from specialized broiler systems and the biggest poultry meat producers are the USA, with almost 20 million tons a year, followed by China, with 18 million tons, the EU and Brazil with about 13 million tons each (16). Poultry meat and eggs are the most common animal source food consumed at global level. Average per capita consumption of poultry meat is still relatively low in Asia, with less than 10 kg per year, twice as less as in Western Europe and 5 times less than in Northern America (17). In summary, statistical data of poultry production continue to confirm economic benefits and perspective of this industry. From epidemiological or public health side, rapid increase in poultry production is coupled with challenges such as environmental impact, animal welfare, antimicrobial resistance and zoonotic diseases. Objective of this paper is to review scientific data, current knowledge and recommendations on poultry production biosecurity and food safety concerns. Our intention is to assess accuracy and efficiency of application of scientifically documented practices and to discuss solutions to enhance both industrial and governmental capacity in prevention of animal health and welfare disturbances and consequent impact on human health and market stability.

Biosecurity, Surveillance and Poultry Production Systems

Biosecurity in farms, zones and compartments is certainly one of the most commonly debated issue in disease epidemiology, national and international animal health regulations and related scientific literature, especially after crises involving transmissible disease such as Foot and Mouth Disease outbreak in United Kingdom, Highly Pathogenic Avian Influenza (HPAI) in Asia and Europe and vector borne zoonosis in Africa. According to Terrestrial animal health code of World organization for animal health (OIE) *biosecurity* is defined as a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population (18). *Biosecurity plan* identifies potential pathways for the introduction and spread of disease in a zone or compartment, and describes the measures which are being or will be applied to mitigate the disease risks, if applicable, in accordance with the recommendations in the OIE Codes (18). The same source defines *animal health surveillance* as a tool to monitor disease trends, facilitate the control of disease or infection, provide data for use in risk analysis (animal or public health purposes), and substantiate the rationale for sanitary measures. The OIE standards (code and diagnostic manuals), as scientifically based policies, are the result of consensus among the veterinary authorities of the OIE members, and those standards constitutes a scientific and legal reference within the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures. The following criteria for listing diseases in the list of reportable (notifiable) diseases are found under Chapter 1.2. of the Terrestrial Animal Health code (18):

International spread while some countries remain free if the disease occur (e.g. Newcastle disease)

Zoonotic potential with human consequences (e.g. *Salmonella*)

Significant spread within naive population with significant morbidity and mortality (e.g. infectious bursal disease)

Emerging disease with zoonotic potential or expanded distribution (e.g. Highly Pathogenic Avian Influenza - HPAI)
The international standards for animal health and zoonosis introduced and promoted application of risk analysis; methodology constituted of hazard identification, risk release, exposure, management and communication in international trade policies. However, principles of risk analysis are applicable in designing and implementation of surveillance and biosecurity plans for animal farms in national frameworks.

According to the new EU animal health law (2016) biosecurity is one of the key prevention tools at the disposal of producers and others stakeholders to prevent the introduction, development and spread of transmissible animal diseases to, from and within an animal population (5). The adopted biosecurity measures should be sufficiently flexible, suit the type of production and the species or categories of animals involved and take account of the local circumstances and technical developments. However, beside this broad legal definition and importance, regulation left degree of power for producers, Member States (MS) or the EU Commission to promote prevention of transmissible diseases through higher biosecurity standards. This means that research of efficiency and effectiveness of biosecurity is still open book and researchers are invited to accept this challenge.

Poultry production systems today are still very heterogeneous and diversified within country and between countries, ranging from small, backyard flocks, and semi intensive and intensive to large fully integrated poultry operation functioning as closed biosecurity compartment. A number of studies investigated association between production system and disease outbreaks and concluded that every poultry farm has its own risk profile for the introduction of pathogens, subsequent development of disease and spread of pathogen to other farms (15; 20; 23). Hence implementation and maintaining of biosecurity measures and plans can reduce but not fully eliminate the risk of pathogen introduction and their consequences. The experience and studies on transmission of highly pathogenic diseases such as HPAI had not showed scientific evidence for distinguishable difference in disease containment among farms of different biosecurity status. In 2004, the Food and Agricultural Organization of United Nations (FAO), analyzing range of poultry production from small or single farm or flock to complex integrated systems, defined four production systems based on the characteristics of the production methods, especially the biosecurity measures implemented, and the extent of involvement of the farm in the formal market chain (7).

Table 1: Summary of poultry production systems (7)

Production system	Main characteristics
System 1	Integrated, industrialized enterprise with sophisticated high-level farm biosecurity measures. Full control over all farm inputs and outputs (e.g. breeding stock, feed mill, slaughterhouse, processing, distribution, animal health services).
System 2	Commercial, intensive poultry production involving largely independent enterprise or contractors, practicing moderate to high-level biosecurity. Distribution of poultry to slaughterhouse and/or to live poultry market.
System 3	Commercial farms with relatively poor biosecurity. Sales are more likely to be through live poultry market or to traders who on-sell through live bird markets. This system covers ducks and other poultry. Production may be intensive or extensive.
System 4	Village - level, scavenging chickens for local consumption. These small flocks are reared in village household. An occasional bird is sold locally, bartered, used as a gift or, occasionally, sold to a poultry trader for cash.

This classification of poultry production systems is valuable for further research of this topic, assuming that systems operated under high level biosecurity are more protective against biological hazards. However, it should be noted that occasionally poultry production operation classified as system 1 can fail to provide biosecurity measures belonging to this system. Form other side; it is possible that farm from system 4, in which no biosecurity is practiced at all, because of external conditions, is more secure than farm from system 1. Sims (2007), reported there is no such thing as a fully biosecure farm and the investment required to achieve this would never make economic sense. Farmers operating large farms have commercial interest to reduce risk of economic damage due to disease introduction. However, their understanding of disease risk or other type of hazards is rooted on industry based priorities (common diseases), for most of which standardized protocols are applied (i.e. immunization, specific pathogen freedom).

Understanding basic principles of biosecurity, shifting disease risks, cost and revenues appears to be low even in farmers operating highly intensive production (14). This is particularly evident in case of need for accounting for emerging and transboundary diseases. For example, HPAI is internationally notifiable disease of significant public interest; hence most of prevention measures are implemented through public systems and funding. They are based on demonstration and ensuring the status of disease freedom for a country, trade restrictions based on sanitary status of trading partners, surveillance including early disease detection and contingency planning to ensure prompt containment. Here farmers hugely depend on public veterinary system outputs (i.e. surveillance systems in place) to exclude hazard abundancy around their operations with biosecurity systems that are not designed and cannot fail safe prevent and contain such level of risk.

Considering current interlinked nature of health risk for humans, animals and environment, scientists are called to support reaching common understanding and develop functional connections between private and public disease management systems (poultry farm biosecurity and surveillance programs) which seemingly have different focus, scope and economic justification. Effectiveness of these endeavors lies not only on scientifically and economically sound solutions, but also in acknowledgment of many other factors that empirically influence cohesion and divergence of motives, capacities and interest of involved stakeholders.

In Australia (20) implementation of biosecurity measures is linked to co funding agreements between government and industry to support handling emergency animal diseases. In some other countries, mandatory biosecurity plans are linked with governmental requirements to operate poultry farm. Even though core for on farm biosecurity and national surveillance plans lies in setting appropriate objectives through hazard prioritization, most veterinarians have not been formally trained in risk analysis (9), although they are expected to use the same scientific principles in surveillance and biosecurity planning.

In developing biosecurity plan, risk analysis methodology is essential. Its first step, hazard identification establishes the self-explicatory link between surveillance and biosecurity. According to Bisdorff et al., (2017) traditionally applied input based surveillance systems (i.e. focused on data collection) led to rigid and divergent requirements towards producers, even in the EU where animal health and food safety issues are of high priority and extensively regulated. Development in surveillance design led to alternative approaches (probabilistic system sensitivity, risk based sampling), where heterogeneity in populations (or production systems) can be more adequately accounted for including various risk levels, simultaneously reducing the cost in achieving the same target. Hence, risk-based strategies and multi-hazard surveillance are more often applied by surveillance and biosecurity programs funded by the private sector (2).

Most of scientists and poultry production experts are in agreement that enhancement of biosecurity measures is most efficient response to mitigate risk of diseases introduction. Scientific debates are still open in answering questions of importance and role of production system in transmission of diseases in connection to disease surveillance. There is need to include biosecurity standards, practices and principles into disease outbreak studies and to enable access of scientists to surveillance and survey data from governmental disease official controls.

Food Safety Concerns

Many definitions, guidelines and standards explain essence of food safety, though, the most commonly it is defined as the system that keeps food and food products free from substances hazardous to human health (6). This paper will be focused on relevant microbiological and chemical hazards.

Food safety, beside governments and producers, receive constant public attention, so different demands and requests by those stakeholders continue to put pressure to scientific community to develop and improve food safety standards. In developed country, regulations and implementation are based on international standards and scientific opinions thus reducing the risk to consumers health on acceptable level. However, in developing world, situation is far behind this. Failure or lack to implement international standards in developing world not only keeps these countries isolated from international markets, but in the same time reduce capacity to prevent health hazards to humans. Global incidence of food borne diseases is influenced by a number of factors grouped in several categories: food supply system, health and demographics, social situation and lifestyle, health system, infrastructure and environmental conditions (12). Intensification of food production, changes in processing, handling and market access keep challenges of food safety system under scientific highlight more than ever.

The most important microbiological hazards affecting poultry meat industry are *Salmonella* spp., *Campylobacter* spp, *Listeria*, *Clostridia*, *Enterococci* and *E.coli*. According to the report of European Food Safety Agency (EFSA) *Campylobacter* was the most commonly reported gastrointestinal bacterial pathogen in humans in the EU and has been so since 2005 (4). The number of reported confirmed cases of human campylobacteriosis was 246,158 with an EU notification rate of 64.8 per 100,000 population. Since *Campylobacter* does not cause clinical signs in poultry, it remains unclear how flocks get infected with *Campylobacter* before harvesting. Feed and water, vectors such as rodents and flies, horizontal transmission between birds, and contamination in the hatcheries are possible routes of entry (8).

Other important food-borne pathogen remains *Salmonella* spp. currently, despite of the success of *Salmonella* control measures, the main sources of infection for humans include meat products, predominantly contaminated poultry meat. In 2017, 91,662 confirmed human salmonellosis cases were reported in the EU. The EU notification rate was 19.7 cases per 100,000 population (4). Reported proportion of human *Salmonella* enteritidis cases increased, mostly due to one EU member state (MS) starting to report serotype data. Sixteen MS met all *Salmonella* reduction targets for poultry, whereas 12 MS failed meeting at least one. The EU flock prevalence of target *Salmonella* serovars in breeding hens, laying hens, broilers and fattening turkeys decreased or remained stable compared to 2016, and slightly increased in breeding turkeys. In recent years, a shift in *Salmonella* serotypes related to poultry and poultry production has been reported in different geographical regions, being particularly associated with the spread of certain well-adapted clones. Moreover, antimicrobial resistance in non-typhoidal *Salmonella* is considered one of the major public health threats related with food-animal production, including the poultry production chain and poultry meat, which is an additional concern in the management of salmonellosis (1). This spread has been facilitated by industrialization and the growing international trade in animal feed, live animals and food. Even though *Salmonella* strains found in human food have been traced back to animal feed (3), changes in consumption pattern may even more influence increasing incidence of human infections.

World health organization formulated a three-step approach to mitigating the risk posed by *Salmonella* spp. (24) and the terms used in this specific case are also relevant for other microbiological hazards. The first step is pre-harvest control, which focuses on the feed and poultry producers. The second step is harvesting control, which covers hygiene measures at the time of slaughter, and third step is post-harvest control, which covers the product from the processing establishment all the way to the end-consumer. It is suggested that the first step, pre-harvest control, is the most important means to prevent infection with pathogens such as *Salmonella*, as traditional control systems are unable to control these pathogens later in the chain.

Considering relation of production systems with incidence of food borne hazards, literature indicated that, compared to chickens in indoor systems, keeping chickens in free-range systems with outdoor access is associated with higher public health and food safety risks for certain hazards, such as *Campylobacter* contamination, avian influenza, and dioxin (10; 13; 19). In a study of Rosenquist et al. (2013) meat from organic broilers was about two times more likely to be contaminated than meat from conventional kept broilers (54% vs. 20%), even though public perception that meat from free range or organic production system is healthier and safer for consumers (22).

The issue of the use of antibiotics as feed additives, pesticides and other chemical pollutants and consequent restriction due to residues found in final products, receive growing attention of scientific community, national and international regulators. Opposite to practice in the EU, antibiotics are still used as growth promoter in order to enhance the production qualities of poultry and other livestock in many developed countries, including USA. Use of pesticide is highly regulated in the EU and in the USA, and residue levels are therefore under strict control. However, in a developing country the situation may be quite different. Disinfective agents used in production establishments and processing plants might be risk factors as well. Chlorinated water used in rinsing the carcasses has also raised concerns among consumers. In the EU the use of chlorinated water is banned, but in the USA it is still a common practice.

Antimicrobial usage and antimicrobial resistance is a complex problem that affect society and is driven by many interconnected factors. Single, isolated interventions have limited impact and coordinated action is required to minimize the emergence and spread of antimicrobial resistance. However, not so long ago, study imply that the benefits of reducing the amount of resistant bacteria by controlling the use of antibiotics as feed additives might be overshadowed by an increase in the number of cases of human food-borne illnesses (21). Some studies have shown that production systems using antibiotics as feed additives achieve growth rates up to 10 percent higher than those not doing so (11). There have been other beneficial effects on the product quality, such as decreased fat and increased protein in the meat, as well as indirect benefits such as a reduction in the amount of feed needed, and therefore a reduction in the amount of waste. It would appear that restrictive policies toward reducing residue level in poultry products reduce long term consequences on human health, but at the same time increase risk of more immediate threats (infection diseases), impairing at the same time poultry production of developing countries in terms of economics or production and international market access. Importance of scientific support to develop new and asses of existing regulation related with this topic remain urgent issue.

International Standards and Market Access Driven Approach

The major international regulators for animal and human health and food safety are the OIE with its Terrestrial Animal Health Code, World Health Organization (WHO), FAO/WHO Codex Alimentarius Commission with its Hazard Analysis and Critical Control Point (HACCP) guidelines, and the World Trade Organization (WTO) which sets the sanitary and phytosanitary (SPS) framework for international trade. The Codex Alimentarius Commission has also set out guidelines for good agricultural practices (GAPs), good manufacturing practices (GMPs) and good hygiene practices (GHPs). These sets of rules and practices are widely accepted in developed countries and international markets. In addition to the main rule setters mentioned above, there are several other international and regional bodies. Regionally, there are organizations such as the EFSA and the African Regional Standardization Organization (ARSO). Regulations set by private industry should also be taken into consideration. These regulations are sometimes more stringent than those described above.

Developed countries follow international standards and use them as basis for adoption of national regulations, however developing world is faced by various challenges to do the same and this situation stay unchanged for years. Market driven approach seems to become most efficient solution for capacity building to implement surveillance and food safety system based on international standards if developing country has export potential. Good example is current practice in the EU that request fulfilment of a number of criteria followed by external audit, before third country can be allowed to export poultry products into EU. In the case of poultry meat, the primary objective of the audit is to asses weather official control system covering the production of poultry meat and products and certification procedures in place can provide adequate guarantees that the production of those commodities is in line with the requirements laid down in the EU legislation and, in particular, are able to support the attestation contained in the relevant export health certificate. The audit is focused on several areas: organization and performance of competent authority, animal health standards, hygiene and public health, official control, monitoring of residues of veterinary medicines, pesticides and contaminants, approval of establishment, organization of diagnostic laboratories and border inspection.

Conclusions

Based on the production growth trends and increasing importance of animal protein worldwide, poultry sector remains the most important livestock industry in satisfying need of human population. In the same time, it is still much diversified in terms of its structure and range of production systems. From epidemiological or public health perspective, rapid increase in poultry production is coupled with various challenges such as environmental impact, animal welfare, antimicrobial resistance and zoonotic diseases.

In the developed world, i.e. the EU, responsibility for food safety shifted from government to producers, and the same shift of policy is supported by international standard setting bodies and national regulations. Strong emphasis is placed on process controls - throughout the food chain, from farm to fork, transparent flow of information and quality management. This philosophy reflects the demands of the consumer and exploits the opportunities opened by technical progress. However, experiences with recent food safety crises, once occurred, demonstrated that public pressure to government will remain high.

Hence, modern production systems and trade structures are now capable of providing full transparency, not only on animal health and food safety issues but also on environmental standards of production and animal welfare. Checks on the end product alone would clearly not be capable of providing the same level of safety, quality and transparency to the consumer. However, challenges to implement international standards in developing countries continue to exist (lack of surveillance programs, deficiencies in administrative, financial and technical infrastructure) keeping increased global risks on animal and human health. The importance of environmental, cultural and political factor must not be neglected as well.
Possible solution for these issues may be found in development of surveillance design (risk based surveillance and sampling) which had already showed to be more flexible, effective and affordable both for public and private sector. Risk approach is also essential in improving efficiency and effectiveness of biosecurity on farms, where more studies are needed to asses and assist in answering question whether transmissible disease on farm happen as results of poor management or weather the level of infections around the farm was such that it overwhelmed otherwise "reasonable" measures. In order to translate current advances in relevant scientific research into practice, it is important to effectively communicate individual and common benefits as well as distribution of costs and responsibilities to all stakeholders. Key responsibility here lies on veterinarians and veterinary sector, which should not only become more susceptible and proactive to solidify their knowledge and skills, but to advocate them to farmers, governments and also other sectors such as public health. Poultry producers will continue to play a vital role in recognizing and monitoring production data and clinical signs (morbidity and mortality) that can ensure early recognition and detection of poultry diseases (passive surveillance), however without control measures in place such surveillance itself, this will not be effective.

Key question remaining unanswered is whether it is appropriate in developing countries to enforce control and regulation systems of the type commonly applied in the developed country. Keeping microbiological hazards endemic, unknown, underreported and uncontrolled, in any part of world, will continue to be biosecurity risk for production in developed countries. Importance of relation, interconnectivity and interdependency of biosecurity, surveillance and food safety system is relatively recently recognized as One Health concept. One health is an approach to design and implement programs, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes. The areas of work in which a One health approach is particularly relevant include the control of zoonosis, food safety systems and combatting antibiotic resistance. Further efforts are needed to implement this concept equally to diverse production systems and two economically and epidemiologically separated area in the world.

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O18 The Rapid Alert System for Food and Feed" (RASFF) to Evaluate the Safety of Poultry Meat Products

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Abstract

European Union (EU) member countries established "The Rapid Alert System for Food and Feed" (RASFF) to ensure the safety of food products imported for their population. The aim of this study was to evaluate the data obtained from RASFF portal during the period of 1992 and 2018 related to the notifications on poultry meat and products. In this search, notification, such as the years, hazard category, risk definition and also notifying country were analyzed. During this period there were about 2664 notifications about poultry meat and products. The most common classifications of notification were alert and border rejection. According to the hazard category classifications, pathogenic microorganisms and their toxins have been the most concerned for poultry meat and products. In addition, *Salmonella* spp., was found to be the most widespread cause of notifications. In order to improve the competing, and enable to import poultry products into EU countries, it is important to evaluate RASFF notification for Turkish poultry industry that is one of the pioneer globally.

Key Words: RASFF, Poultry meat and products, Food safety, European Union, Export

O[19] Effects of Different Concentrations of *Laurus Nobilis* Essential Oil on *Escherichia Coli* ATCC 25922 in Broiler Meat

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Abstract

In this study, the inhibitory effect of different concentrations of *Laurus nobilis* essential oil on *Escherichia coli* ATCC 25922 was investigated by spreading plate method. Natural microflora of chicken breasts were removed and 240 µl *Escherichia coli* ATCC 25922 and 5 different concentrations (160, 200, 240, 480 and 2400 µl) of *Laurus nobilis* essential oil were mixed and applied on samples. Microbial load of *Escherichia coli* ATCC 25922 culture which is inoculated to the chicken breast was determined. After this inoculation procedure, samples were kept at +4 ◦C for 0, 1, 3, and 5 days. After this incubation period microbiological analysis were conducted and microbial load of each sample was determined as cfu/g. As a result of the experiment, it has been found that all concentrations of *Laurus nobilis* essential oil were decreased the microbial load and the maximum inhibitory effect of *Laurus nobilis* essential oil on *Escherichia coli* ATCC 25922 was obtained at the volume of 2400 µl.

Keywords: *Laurus nobilis* essential oil, *Escherichia coli* ATCC 25922*,* chicken breast meat.

O[20] Disinfection Recommendations against *Salmonella* Typhimurium and *Salmonella* Enteritidis Biofilms in Poultry Meat Industry

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Abstract

Disinfectant agents' efficacy were evaluated for against *Salmonella* Typhimurium and *Salmonella* Enteritidis biofilm, can be used in poultry meat industry in Turkey. In this context, two different S. typhimurium, S. Enteritidis isolates, isolated from the 'Monitoring and Control Programs of *Salmonella* from Poultry and Foods' project and S. typhimurium ATCC 14028, S. Enteritidis ATCC 13076 strains were used for the biofilm formation, and then disinfectants used in the study were investigated for the efficacy of these biofilms. The disinfectants used in the study were determined as peroxyacetic acid, quarterner ammonium compounds, alcohol-containing disinfectants and sodium hydroxide solutions, which are frequently used in poultry meat industry. In this study, 13 different sub-experimental groups were formed, these groups were prepared by the concentrations of 10^3,10^5 and 10^8 cfu/ml of four different microorganisms used in the study. The microorganisms used in the study were classified as A (S. Typhimurium wild type), B (S. Enteritidis wild type), C (S. Typhimurium ATCC 14028), D (S. Enteritidis ATCC 13076) groups. The negative control group was named as K. According to the results of the study, it was concluded that the efficacy of the disinfectants used against S. Typhimurium and S. Enteritidis biofilms was directly related to the concentrations of the microorganisms that formed the biofilm. On the other hand, the resistance profiles of S. Typhimurium and S. Enteritidis biofilms differ to disinfectants used. It is observed that S. Typhimurium biofilms are more resistant to disinfectant treatments containing alcohol. The effect of peroxyacetic acid on the inhibition of biofilm formation is quite high compared to other disinfectants. Results showed that the biofilm counts after peroxyacetic acid treatments, were below the detection limit for all biofilm groups except those of the group D biofilms with a concentration of 10^8 cfu/ml. After this disinfectant treatment, all concentrations of both *S*. Enteritidis biofilms remained below the detection limit. Considering the results of the study, it is thought that the use of PAA can be included in sanitation procedures in order to prevent *S*. Typhimurium and *S*. Enteritidis biofilms in in poultry meat industry.

Keywords: *Salmonella*, biofilm, disinfection, peroxyacetic acid, benzalkonium chloride

O[21] The Effect of Edible Film Coating Carrying Antimicrobial Properties on Shelf Life Extension of Chicken Breast Meat

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Abstract

As a consequence of growing environmental problems due to plastic use, investigations on nature-friendly coating materials have been intensified in recent years. Due to its lower cost and degradable nature; starch based polymer coatings are more preferred. Although different ingredients such as casein, whey protein concentrate, gelatin etc. are used for the production of edible film, studies related to production of edible film from potato industry effluent is very limited. Therefore, production of edible film from potato industry effluent, and its ability to extend shelf life chicken breast was prompted in this study. Chicken breast samples were coated with edible film, stored at 4 °C, and the samples were analyzed on 0, 3, 5 and 7th day. pH of the samples at the beginning of the shelf life study was measured as 5.58 ± 0.02. While uncoated samples had a pH of 6.56 ± 0.03 at the end of the shelf life study revealing a significant increase in seven days, pH of the coated samples had pH of a 5.67 ± 0.02 with insignificant change. Initial number of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) were counted as 3.04 log cfu/g and 2.17 log cfu/g. After seven days of storage both TAMB and TMY of the control samples were increased to 6.11 log cfu/g and 7.5 log cfu/g; whereas coated samples had TAMB and TMY counts of 4.8 log cfu/g and 5.13 log cfu/g, respectively. It was concluded that edible film produced from the potato industry effluent can effectively be used to coat chicken breast samples to increase shelf-life.

Keywords: edible film, chicken breast, shelf life extension, antimicrobial film

Introduction

Poultry meat is a very popular food commodity around the world, and its consumption has increased over the last decades in many countries. Some of the reasons for the popularity are the relatively low cost of production, low-fat content and the high nutritional value of poultry meat. (Soyer et al.,1999). The shelf life of packaged fresh meat is very short due to rapid microbial growth and oxidative stress. Various processing methods like freezing, drying, addition of chemical preservatives, and vacuum as well as modified atmospheric packaging are employed to ensure safety and quality of meat (Zhou et al., 2010). The primary objective of meat packaging is to reduce and/or inhibit the growth of spoilage and pathogenic microorganisms and minimize the chemical deteriorations by inert and passive containment. In addition, different packaging materials can be designed to improve food quality by adding active ingredients to the packaging material. These active ingredients can help extend the shelf life of meat, enhance sensory qualities and extend shelf life (Zhou et al., 2010). Other different approaches such as biosensors and plant essential oils are also used to extend the shelf life of meat (Bozkurt, 2006; Pokorny, 1991).

There are different studies on the use of coating materials to extend the shelf life of chicken meat (Ramakrishnan et al., 2018; Ahmed et al., 2018; Mahdavi et al., 2018). Some of these coating materials are; edible films, biopolymers, and biofilms. Biodegradable edible films are used to limit moisture, aroma and lipid migration in foods where conventional packaging is insufficient. In addition, components carrying antioxidant properties and antimicrobial properties and coloring agent can be added to the edible films to provide microbial protection (Torlak and Nizamoğlu, 2009; Souza et al., 2018; Mali and Grossmann, 2003). Even though studies related to shelf life extension of poultry products with edible film from different sources were conducted; shelf life extension of chicken breast meat with edible film from the effluent of potato industry is not reported. Thus, this study is conducted to determine the potential of edible film from potato industry effluent on shelf life extension of chicken breast meat.

Materials and Methods

Materials

Chicken breast samples were purchased from the local markets (Bolu, Turkey). Effluent from the potato factory was taken from Köksal Patates Sanayi ve Ticaret A.Ş. (Bolu, Turkey). Dried leaves of *Origanum onites* were also purchased from local markets (Bolu, Turkey).

Methods

Preparation of edible film: Natural sedimentation was carried out at room temperature for 24 hours to separate the effluent from the potato pieces, skin and other particles. Then the upper part of the effluent was centrifuged at 240 RPM for 5 min, the pellet was dried for 24 h at 37 °C, and sieved to obtain starch in fine granules. Twenty-five g of dried starch was mixed with 250 mL of distilled water and 17 mL of glycerol was added. The mixture was stirred at 190 RPM for 90 min (Wisestir, Germany). After 3 minutes of stirring, 30 mL of HCI and 150 µL of yellow color food grade colorant was added. Closer to the end of the stirring process 30 µL of oregano essential oil was added, and obtained film was allowed to cool for 15 min at room temperature.

Coating of the chicken breast samples: During edible film formation temperature was decreased to 60 °C during last 15 min, and the chicken breasts were plated by formed gel with the dipping method. After coating, the samples were allowed to dry for 10 minutes at 22 °C. At the end of the drying, the samples were placed in Ziploc bags®, bags were locked and stored in a refrigerator at 4 °C.

Microbiological analysis of the chicken breast: Total mesophilic aerobic bacteria (TMAB), total mold and yeast (TMY), and total coliform (TC) counts were performed.

Measurement of color properties: The pH of the control and coated samples were measured using a SELECTA pH-2005 pH meter. The color of the samples was determined using a colorimeter (Konica Minolta CR-400, Osaka, Japan). Values of the Hunter scale were expressed as L^* (0 black to 100 (white), a^* (-greenness to + redness), b^* (-blueness to + yellowness) (Francis and Clydesdale, 1975) and total color difference ΔE was calculated.

Data analyses: Statistical data analyses were performed using Minitab 17 (Minitab, Inc., State College, PA). One-way ANOVA with Tukey multiple comparison test was performed at 95% confidence interval.

Results and Discussion

The initial pH of fresh chicken meat was 5.58±0.02 at the beginning of the shelf life studies and it increased during storage period at 4 °C for all uncoated samples. There was no statistically significant difference in the pH values for the first 3 days but pH increase was statistically important after 3 days ($p > 0.05$). At the end of the 7-day storage pH of the control samples increased to 6.56 ± 0.03 ($p \leq 0.05$). film coated samples on the other hand had more stable pH, and the pH of the samples after 7-day storage was recorded as 5.67 ± 0.02 revealing no significant difference ($p > 0.05$) (Fig. 1).

In their study Sogut and Seydim (2018) concluded that the pH of the film-coated chicken meat samples with initial pH of 6.8 was stable after 12 days of storage wıth antimicrobial packaging. Although the initial pH of the samples was different, it is found that the antimicrobial coating provides pH stability during storage at 4 °C.

Color L^*, a^* and b^* values of the control chicken breast were 77.46±2.27, -1.31±0.25 and 9.05±0.90 with ΔE of 20.66±2.22, respectively. At the end of storage L^*, a^* and b^* values for control samples were 31.03±2.25, 3.42±1.42 and 7.42±1.01 consequently. ΔE of the control samples at the end of the 7-day storage was measured as 64.17±2.16 ($p \leq 0.05$). Film coated samples had the color L^*, a^* and b^* values of 86.44±2.14, -2.14±1.16 and 11.795±0.95 at the beginning of the storage. These color values changed to 52.68±3.53, -0.96833±1.01 and 7.80±1.90 after 7 days, respectively. ΔE of the coated samples at the beginning of the storage 16.42±2.13, on the other hand, changed to 43.28±2.97 revealing significant difference (Fig. 2).

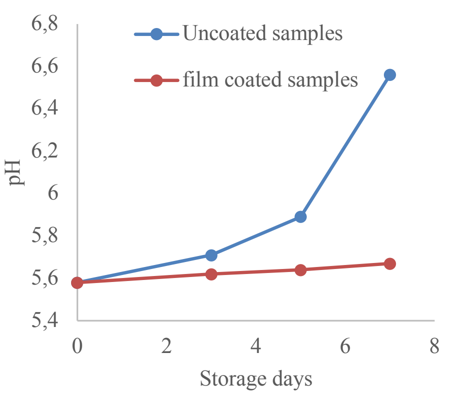

Figure 1: pH change during storage of chicken breast samples at 4 ^{0}C.

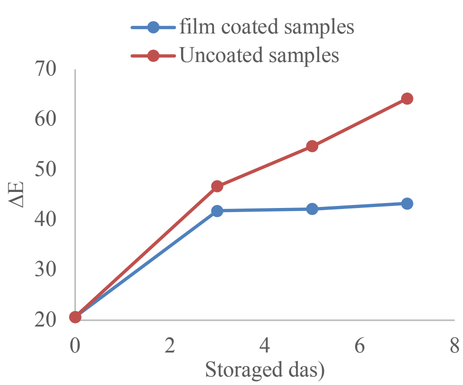

Figure 2: The color difference of the chicken breast samples during storage.

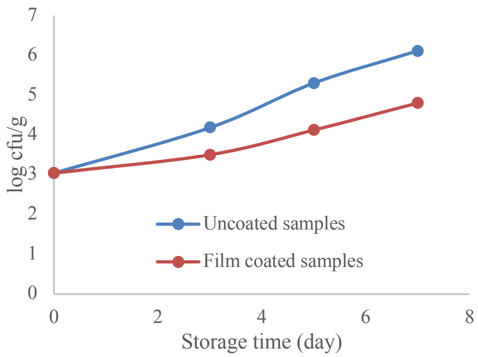

Figure 3: TMAB change during storage.

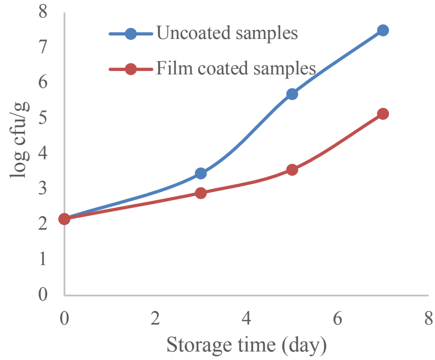

Figure 4: TMY change during storage.

Color is a very important factor for coating materials is because it may affect the color of the food over time affecting physical and sensory properties of the food coated. Thus, edible film produced in this study was opaque-yellowish color compatible with chicken breast. Even though color of the edible film was very close to chicken breast at the beginning of the storage, there was a decrease in the *L** value of the coated samples through the end of the7-day storage. It is possible that the reason for the decrease of *L** at the end of the storage is due to the transition of the surface moisture to the polymer coating (Soysal et al., 2015).

Initial TMAB of 3.04 log cfu/g at the beginning of the shelf life increased to 6.11 log cfu/g for uncoated and 4.8 log cfu/g for the coated samples (Fig. 3). Initial TMY count of 2.17 log cfu/g, on the other hand, increased to 7.5 log cfu/g for uncoated and to 5.13 log cfu/g for coated samples at the end of the 7-day storage, consequently (Fig 4). It was found that coating of chicken breast samples enriched with *O. onites* was effective to extend shelf life by preventing increase of initial microbial load.

Conclusions

Edible coating of food samples is gaining popularity due to extension of shelf life with or without addition of antimicrobial agents, making food more appealing with addition of coloring and flavoring agents, increase nutrition value of food with addition of vitamins, minerals and bioactive compounds as well as functional components and reduction of plastic use. Usually, edible films are made from food components such as casein, gelatin, and whey protein concentrate, and thus limited number of studies are reported production of edible film food industry waste or effluents. This is one of the first report involving both production of edible film from potato industry effluent and coating of chicken breast samples with the edible film produced. It was observed that coating of chicken breast samples with the edible film provided shelf life extension of chicken breast samples. Future studies need to involve detailed quality analyses of chicken breast samples during shelf-life studies.

Acknowledgements

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IS[12] Vaccination Against ILT, Field Conditions and Challenges for Success in Broilers

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Abstract

Infectious Laryngotracheitis (ILT) is a serious respiratory disease of chickens. Infections occurring in susceptible (unvaccinated) broiler flocks can result in severe disease and high mortality. ILT was a sporadic disease in Australian broiler flocks until about 2007. Since then, recurrent (and often intractable) outbreaks have occurred in the more densely poultry populated areas, such as the Sydney basin (western Sydney and NSW central coast region) and the Mornington peninsula in Victoria. A recombination of two vaccine strains (classified as Classes 1 and 7) resulted in the emergence of multiple classes of recombinant viruses (initially called Classes 8 and 9) which became the predominant outbreak strains. Vaccination in broiler flocks is only undertaken in the face of a local outbreak and, because of the large numbers of birds involved, requires mass vaccination techniques (i.e. via drinking water). While other live poultry vaccines can be successfully administered in this way, ILT vaccines pose a particular problem. ILT virus requires contact with respiratory tissue (conjunctivae, nasal cavity, trachea) to adequately infect and immunize the bird. Contact with only the mouth, tongue and oesophagus is ineffective. Hence water administration of ILT vaccine requires accidental contact of the vaccine water with respiratory tissue. This can be highly variable between flocks. Water vaccination with ILT in broilers is plagued by vaccine reactions and in some cases, wild strain outbreaks in vaccinated flocks. The effectiveness of the vaccine is often questioned in the latter cases. Studies were undertaken to attempt to understand the dynamics of water vaccination in the field. An initial study phase showed that vaccine uptake by individual birds from water administration was highly variable and relied upon bird to bird transmission to effectively spread the vaccine virus through the flock. This spread phenomenon would explain the rolling vaccine reactions sometimes observed. Some flocks did not take up the vaccine at all well and the resulting slow spread of vaccine virus through the flock left a large number of birds susceptible at later ages when wild virus incursion was more likely. The latter situation explains some apparent vaccine failures. A number of vaccination procedure factors were identified as contributing to the relative success of vaccine application from this study and a further, more extensive study, using detection of ILT vaccine virus in dust was undertaken. The large study hoped to validate the effects of the type of water stabilization used (skim milk products compared with commercial dye stabilizer), the time that water was stabilized prior to vaccine addition and possibly, source of the chicks.

This study is still under way but preliminary results are tending to confirm better results using skim milk products and longer time of water stabilization as assisting vaccine uptake. Some other factors may emerge as important as well, including shorter times for vaccine consumption and administration of vaccine earlier in the morning, but these are preliminary findings only. Completion of the study will be required to make valid conclusions on these effects.

The outcomes highlight the importance of assessing vaccination dynamics in large flocks of young meat birds and new technology has enabled a better appreciation of this in the field. An economical, rapid test for determining vaccine success is now possible using a single dust sample at a determined time post vaccination appears possible. Determination of advanced methods of improving vaccine delivery is under consideration. Further studies to validate the identified factors and to identify further issues are necessary.

Introduction

Infectious Laryngotracheitis (ILT) is a serious respiratory disease of chickens. The causative agent is an alphaherpesvirus which infects the upper respiratory tract, causing marked conjunctivitis, rhinitis and haemorrhagic tracheitis. Morbidity can reach 100% in susceptible flocks and mortality can vary up to as much as 70%, although most field outbreaks in broilers reach about 15% mortality in Australian experience. Vaccination of broilers against ILT is only undertaken in the face of a local outbreak of the disease. The vaccines which have been developed mainly for protection of long lived birds (commercial layers and breeder flocks) generally provide reasonably severe reactions in young broiler chickens.

Mass vaccination in young meat chickens using live ILT vaccines can be associated with so called "vaccine reactions", where clinical signs of ILT may manifest in the vaccinated flock; and evident failure of vaccine to protect against incursions of wild strains. When tested in the laboratory however, these wild strains generally are shown to be protected against [1, 2, and 3]. When examined in a laboratory experiment, differences are seen between eye drop inoculation and drinking water administration in the timing of viral replication in tracheal cells [4,5,6]. It is obvious that bird to bird transmission is a major occurrence when the drinking water route is used, even within closely controlled laboratory administrations.

ILT vaccines are generally recommended to be given by the eye drop route. Because of the large numbers involved with broilers however, mass vaccination techniques are the only practical option. This usually means application via drinking water. It has been well demonstrated that ILT vaccine virus must invade a respiratory tissue to allow viral replication and hence inaugurate immunity [7].

However contact only with non-respiratory tissues (mouth, tongue, pharynx, oesophagus or gastrointestinal tract) is non-productive and evokes no protection. Drinking water administration only results in minimal respiratory tissue contact. Other reports [8] note that an increased vaccine dose rate, perhaps 10 to 100 fold, needs to be supplied for the best outcome from this route of administration. This would be impractical and uneconomic to achieve.

An initial field study has been reported [9] where individual tracheal swabs were collected from 40-70 birds across 8 broiler houses during a natural ILT outbreak involving a Class 9 wild strain [10]. The Nobilis ILT vaccine, (Serva strain, Class 7, MSD) was used in the face of this outbreak, administered in drinking water between 7 and 14 days of age in broiler flocks. This revealed very poor and inconsistent initial uptake of the vaccine virus from the actual administration and that bird to bird spread of the vaccine virus within the house accounted for the establishment of immunity. The rate at which this spread occurred determined whether the flock would have sufficient immunity at the likely time of wild virus incursion. Three of the 8 flocks would not have been considered protected sufficiently in this study [9]. It was also shown that sampling of dust from settle plates could be used as a useful measure of vaccine uptake. A strong and significant correlation was found between tracheal ILTV detection from trachea (the gold standard) and detection from dust samples at various ages (Pearson R= 0.77, P<0.05), for dust at 7-8 days and trachea detection at 7-8 days post vaccination [11].

The present study looks at the detection of ILT vaccine virus detection in dust from settle plates from broiler flocks following drinking water application performed in an Australian context. The ongoing study takes place during a naturally occurring outbreak of ILT in broilers where the wild outbreak strain as identified as Class 9. Class 9 was found to be a recombinant strain of two ILT CEO Vaccines, Class 1 and Class 7 [3,12,13,]. Class 9 has become the predominant outbreak strain in Australia since 2008 [10].

Materials and Methods

A natural outbreak in the Sydney basin involving ILTV Class 9 began in June-July 2018. The study aims at sampling a total of 40 houses across 20 farms within the outbreak area, using dust samples in each house collected on settle plates on days 4, 7, 14 and 21 post vaccination (pv). Results from 24 of these houses are available at time of writing. Vaccination procedures were documented for each house taking part in the study. Vaccination was administered in drinking water using either skim milk (powdered or liquid) or a commercial blue dye stabilizer product (Vac-Pak-Plus, Animal Science Products Inc, Texas, USA). DNA was extracted from the dust samples at the University of New England (UNE, Armidale, NSW, Australia) for qPCR to enumerate ILTV genome copy number [14]. Positive samples will be determined for ILTV Class at Birling Avian Laboratories (BAL, Bringelly, NSW, Australia).

Statistical analyses were conducted using STATISTICA v6 software comparing prevalence across days post vaccination (pv) and vaccination administration factors using correlation and contingency table analyses.

Results and Discussion

Robertson and Egerton (1981) [7] showed that immunity to ILT required replication of the vaccine virus in respiratory tissue and that following drinking water application, only around 24% of birds accidentally induced contact of vaccine laden water with a target respiratory tissue. Hence we assume that birds which do not reveal the presence of ILTV in their trachea will not have mounted an immune response to the virus. We expected that peak viral copies detected from the trachea should occur around day 8 pv (from laboratory studies [4,6]. The initial study [9] revealed a very variable detection of ILTV following vaccination. All virus detected in this study was found to be of vaccine type (Class 7) and no wild isolates were detected in this study. Actual uptake of the virus from the vaccination application was generally inefficient. The highest prevalence of ILTV detection from tracheas at 4 days pv was 52% of birds and this extended to as low as 3% in some flocks [9]. The study indicated that considerable bird to bird spread of the vaccine virus was occurring and that establishment of sufficient house immunity would be reliant on this method. As reversion to virulence with continuous passage between birds is a known feature of ILT vaccines [15], this finding possibly explains the observed "vaccine reactions" reported frequently from the field. The slowness to reach a high prevalence of birds with detectable ILT vaccine in their tracheas in some houses would indicate there were large numbers of susceptible birds remaining at later ages and these may explain the observed apparent failures of vaccination reported [9].

This initial study involved only a small sample size (only 8 flocks) and analytical interpretations may be inaccurate. However a couple of interesting associations between the level of vaccination success and some vaccination procedures were observed. "Poor" takes were associated with the use of the dye stabilizer product as compared to skim milk products [9] and this was somewhat corroborated by the lower ability to isolate ILTV from the drinking water stabilized with dye than from water stabilized with skim milk [11]. It was also indicated that longer time of water stabilization (time between addition of stabilizer to the drinking water and addition of the vaccine) was associated with the "Better" take flocks [9]. There was also a possible association with the source of the chicks (P=0.07) with prevalence of tracheal ILT DNA detection at 12-13 days. This could have involved factors with the hatchery, breeder flocks, breeder flock age or transport. These findings however needed verification.

The present study was based on the above findings and was conducted to attempt to verify them and to seek other possible factors that may be able to be used to modify vaccination outcome. Collection of two dust samples per house at 4, 7, 14 and 21 days was undertaken using settle plates which were cleaned after each collection. Thus the plate contained dust deposited freshly on the plate only between the collection times. At time of writing, results from 24 houses of the designated 40 are available. Results are hence only preliminary. It should be noted that the personnel applying the vaccine followed the standard operating procedures recommended by the vaccine manufacturers and company veterinarians very closely, with all the important aspects of water deprivation, rapid availability of vaccine containing water and activation of the flock to encourage drinking being assiduously observed.

So far there appear to be no statistically significant associations with broiler house style (i.e. conventional compared to tunnel ventilated), number of birds per house, date of vaccination, actual vaccine doses administered per bird (within a very small range close to 1 dose per bird), the amount of stabilizer used or the volume of water allocated per bird for vaccination. However, the length of time of water stabilization (i.e. time between addition of the stabilizer to the drinking water and the addition of the vaccine) is showing a moderate positive correlation with dust viral copy number (VCN) at 4, 14 and 21 days pv (Pearson R=0.60, P=0.023 at day 4 pv). Also a moderate negative correlation has been observed with total time to consume the vaccine with dust VCN's at 14 and 21 days pv (Pearson R=-0.57, P=0.009). There are also early indications that flocks using the dye preparation have lower dust VCN's at days 4, 14 and 21 than flocks using skim milk products (repeated measure ANOVA means $\log_{10}$ VCN of 3.70 for dye and 5.49 for skim milk, P=0.06), which is trending towards confirming the indications from the initial study. There is some indication that administering vaccine earlier in the morning is associated with higher dust VCN's. Shorter times for the birds to completely consume the vaccinated water may also be related to better vaccine viral detection in dust samples after 14 days pv. Further analyses are required to confirm these relationships.

Conclusions

Unlike many other poultry vaccines, live CEO ILTV vaccines are difficult to administer via drinking water [16]. While other live poultry vaccines, such as infectious bronchitis or Newcastle disease viruses, can effectively vaccinate a bird through contact with the gastrointestinal tract, ILT virus requires contact with its target tissues (respiratory tissue) to be effective. This contact tends to occur accidentally while the bird is drinking. The present studies have shown that in the field situation that this is quite inconsistent even when accepted administration techniques are closely followed. There may however be other factors and procedures which may be able to modify the level of success in vaccination. The dye product used here may be suitable for other vaccines but may not support effective vaccination with this ILT vaccine when used under these conditions. A longer water stabilization time using the dye may improve this, but this would need to be confirmed. There are early indications that increasing the time of water stabilization with skim milk products (powder or liquid) may improve early vaccine uptake and decreasing total water volume used for vaccination so as to reduce the time to consume it may also be helpful. Other factors may emerge as the study continues.

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O[22] Molecular Epidemiology of Avian Influenza (H5N1) Isolates Isolated From Black Sea Region

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Abstract

In this study, molecular characterizations of Hemagglutinin gene of Avian Influenza (AI) H5N1 isolates which was seen in our country in 2006 and reported OIE were made. By this means, the objective of the study was to find out the pathogenicity levels and whether they had the ability to be transmitted from person to person. Within the context of this study, 27 AI H5N1 isolates isolated from various poultry were used. After isolates were revived in chicken eggs with specific pathogen free embryo, chorioallantoic fluids of the eggs were collected and RNA extraction was made from these fluids. Following RNA extraction, first Real Time RT-PCR was conducted on especially H5 sub type with specific primers and probe and later enzyme cut-off area and receptor binding area of hemagglutinin gene were reproduced with RT-PCR. As a result of our study, positivity was found in 17 of the 27 isolates we had and all of them were found to have High Pathogen Avian Influenza Virus (HPAI). In addition, as a result of sequence analysis of receptor binding area, no mutations were found in the related amino acid positions of these 17 isolates and it was concluded that the isolates we had did not have the feature to transmit from person to person; however, due to the character of the virus and the fact that it is still circulated in some places of the world according to latest data and since it is a virus that can be transmitted to migratory birds, it is necessary to determine molecular and antigenic characteristics of the virus in places where the disease is seen in terms of following the potential of AI H5N1 virus to transmit to human beings in the future.

O[23] Metagenomic Analysis of Gut Microbiome Associated With Necrotic Enteritis in Broilers

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Abstract

This study was conducted to determine gut microbiome and establish associations between broiler chickens with and without necrotic enteritis (NE). Gut microbiome of 60 NE-suspected and *C.perfringens* positive chickens from 12 epidemiologic units and 50 NE-free chickens from 10 epidemiologic units were detected by next generation sequencing and metagenomic analysis. There were significant differences between the relative abundance of phylum, family and genus in chickens with and without NE. Significantly higher levels of *Proteobacteria* (%61.6>7.29) and lower levels of *Bacteroidetes* (%0.63<33.6) were detected in NE animals when compared with non-NE animals. A total of 24 families were detected in NE samples and 40 in non-NE samples, 20 being common in both groups. *Enterobacteriaceae* (%55.05>4.31) and *Clostridiaceae* (%23.07>10.36) families were abundant in NE samples, whereas *Ruminococcaceae* (1.27<31.46), *Lachnospiraceae* (0.83<10.27) and *Bacteroidaceae* (0.19<12.98) families were more scare in normal samples. Beside to these classically established taxons, new families like *Rikenellaceae, Porphyromanadaceae, Desulfovibrionaceae* and Erysipelotrichaceae; and new species like [*Clostridium spiroforme*] and *Paeniclostridium sordelli* [*Clostridium sordelli*] were associated with NE.

O[24] Vaccination – The Key to *Salmonella* Control in Poultry

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The number of human food-borne infections with *Salmonella* increased enormously in the late 1980s. The increase was almost exclusively due to *Salmonella* Enteritidis cases. This pandemic created awareness for *Salmonella* in Europe and lead to the implementation of a European *Salmonella* monitoring and control programme. Data from the WHO and the EFSA clearly shows, that the majority of human *Salmonella* cases are linked to the two serotypes *Salmonella* Enteritidis and *Salmonella* Typhimurium. The consumption of poultry meat and eggs, which represent a major source of affordable high energy protein for much of the world, is believed to be the main cause for *Salmonella* infections in humans.

Around 70 % of human *Salmonella* Enteritidis cases were linked to the consumption of eggs, egg products and bakery products. In broiler flocks and broiler meat also *Salmonella* Infantis is found at a high rate with a prevalence of up to 70 % in some EU countries. This clearly illustrates the importance of controlling *Salmonella* Enteritidis, *Salmonella* Typhimurium *Salmonella* Infantis in poultry to be able to reduce human food-borne infections with *Salmonella.*

To reach this goal, a combination of different tools should be used. Proper hygiene and disinfection, rodent and insect control, as well as good farming management practices are a very important part in the *Salmonella* control strategy. On the other hand, a special Commission Regulation (1177/2006) has been issued, that prohibits the use of antimicrobials for the control of *Salmonella*. Live vaccination of chickens to protect against *Salmonella* Enteritidis field infection is of utmost importance in Salmonella control strategies. Live attenuated vaccines derived from Salmonella Enteritidis are widely used against *Salmonella* Enteritidis and *Salmonella* Typhimurium infections, and their efficacy, ease of use and excellent safety under field conditions has been proven. In additional studies the cross protection of a live *Salmonella* Enteritidis vaccine against a *Salmonella* Infantis challenge has been shown. Live *Salmonella* vaccines produce better protection than inactivated vaccines due to the fact that they help to prevent organ colonisation as early and as effectively as possible and reduce shedding and spreading of *Salmonella*. Their ability to give cross protection to multiple serotypes is another advantage of live vaccines. In their joint effort, the poultry meat and egg industry and the authorities have made significant progress in reducing the contamination rate of poultry flocks and products during recent years in Europe. The vaccination against *Salmonella* was made obligatory in laying hen flocks in member states where the *Salmonella* Enteritidis flock contamination was above 10%. Since the introduction of obligatory vaccination of the laying hens, the number of confirmed human cases of human Salmonellosis has drastically declined. This was also confirmed by the EFSA stating that the reduction of *Salmonella* Enteritidis in laying hen flocks and of *Salmonella* spp. in table eggs has contributed to the decline of S*almonella.* Enteritidis cases in humans, since eggs are regarded to be the most important source of these infections. Increased voluntary and compulsory vaccination of laying hens, as well as other hygiene-based control measures are likely to have contributed to this positive development in consumer safety.

As *Salmonella* wild type strains are present in many live and unenlivened vectors like rodents, insects, wild birds, dust, water, feed, litter and many more that can easily introduce field infections into unprotected flocks, these flocks should always be protected by vaccination. This is also true in times of decreasing *Salmonella* prevalence in poultry flocks, as it is impossible to eradicate *Salmonella* from the environment. Besides the success of the European *Salmonella* control programme, *Salmonella* Enteritidis and *Salmonella* Typhimurium still account for the majority of food-borne infections in humans and the prevalence of *Salmonella* Infantis in broiler flocks and at slaughter remains at high levels. Currently we are also seeing a rise in cases of infections with monophasic variants of *Salmonella* Typhimurium. Because of the recent enormous rise in human cases infected with this serotype this serotype has come into focus and considered as the onset of a new S*almonella* pandemic. The broad spectrum of antibiotic resistance found in these strains requires taking them seriously. The emergence of new *Salmonella* is a constant threat. Recently a multi-country outbreak of *Salmonella* Enteritidis phage type (PT) 8 possibly linked to eggs, is ongoing in the EU. Such observations underline the importance to use such *Salmonella* live vaccines for which a broad spectrum of cross-protection against several phage types within their serotype and also across different serotypes has been shown. Such less-attenuated, more vital live vaccines exist and are available in Europe to offer the best possible *Salmonella* protection ensuring peace of mind for the poultry industry and safe poultry products for the consumer.

Progress in layer breeds enable increased laying periods and low egg prices make longer production cycles more attractive to the egg producer. A critical factor in keeping birds for such a long time is to ensure that they remain immune against important diseases, including Salmonella Enteritidis and Typhimurium. For this reason a Salmonella live vaccine should be chosen that is less attenuated and can offer longer and broader protection than more attenuated vaccines. The use of attenuated live vaccines that are based on zoonotic agents in food-producing animals requires high standards for the safety of such vaccines. Besides its broad protection and long duration of immunity all safety criteria have been successfully fulfilled by the less attenuated vaccine mentioned before. To stimulate a proper immune response in the vaccinated chickens which is a prerequisite for protection the vaccine strain has to colonize the birds which will lead to shedding of the strain. For this reason only those live vaccines are allowed to be used in Europe for which a method to differentiate vaccine strains from field strains is available. The detection of *Salmonellae* mostly is based on standardized cultural methods but may also be conducted with validated molecular methods that are used increasingly. The potential re-isolation and detection of SE live vaccine strains rather than field strains is a challenge to the diagnostician. So there is a need for methods capable of distinguishing between vaccine and field strains. There are cultural DIVA – Differentiating Infected from Vaccinated Animals – methods in place, but they are time consuming and need viable isolates. Recently a real-time PCR based detection method has become available which is capable to separately detect SE field and SE live vaccine strains in one reaction within 2 -3 hours after pre-enrichment.

IS[13] Harvesting Market Age Broilers

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Abstract

The harvest of market age broilers accounts for a very short period of the broilers lives but can have significant impacts on yields and carcass quality. Harvesting factors typically include catching, loading, transportation, and lairage. Methods for catching and loading have a major influence carcass damage, DOAs, and broiler welfare. Catching crew training is a crucial component of minimizing damage and improving welfare. Transportation and lairage can have a large influence on DOAs depending on the environmental conditions during these times.

Introduction

Broiler welfare during catching is inherently linked to improving profits in the processing plant. Minimization of broiler stress and injury during catching, loading, transportation, and lairage at the processing plant directly leads to improvements in yield and carcass quality. Stress and injury can lead to DOAs (dead on arrival), carcass damage, and partial or whole carcass condemnations.

When broilers are not treated humanely they can experience pain and fear which can then lead to death, exhaustion, bruising, dislocated and broken bones, torn skin, and general stress. These physical injuries can lead to condemnations, downgrading, and sometimes total loss of the carcass. It has been estimated that about 90% of downgrading due to physical damage occurs in the 12 hours prior to slaughter (Elson 1985). Various studies have reported the impact of catching on carcass damage. Catching of broilers for loading accounted for 32% to 38% of leg and wing bruising as well as 11% of breast bruises (Reali 1994). In another study, the physical condition of the broilers including broken wings and legs, skin lesions, feather cleanliness, and blood was examined both pre and post catching (Jacobs et al. 2017). Jacobs found that catching leads to a significant increase in the percentage of broken wings when compared to broilers prior to catching.

Feed Withdrawal

Optimizing broiler feed withdrawal is a delicate balance between maximizing utilization of feed consumed and clearing the alimentary tract to minimize contamination during processing. If time off feed is too short, unused feed will remain in the alimentary tract. Growth potential from the consumed but not utilized feed will be lost. Carcass contamination will increase due to a greater quantity of feces present during transport and lairage as well as crop and intestinal tearing/leakage during processing. If feed withdrawal time is too long, birds will utilize back up resources after nutrients have run out leading to live shrink and lost yield. Carcass contamination will also increase due to increased likelihood of intestinal tearing.

Water access after feed withdrawal is also an important consideration. During this time water consumption is necessary for crop emptying and provides an opportunity to administer water treatments to birds, which may minimize the presence of pathogens such as *Salmonella* or *Campylobacter* during processing. A commonly used method of water treatment is drinking water acidification. During feed withdrawal, as the crop empties there is a decrease in growth of lactic acid producing bacteria leading to an increase in crop pH. This increase in crop pH allows for the growth of pathogens such as *Salmonella*. Providing acidified water during feed withdrawal keeps the crop pH low, therefore, avoiding pathogen growth. Drinking water can be acidified using products containing organic acids such as lactic, formic, or citric acid or using inorganic acid products containing sodium bisulfate. Although acidification has a positive impact on minimizing contamination, there may be negative effects including damage to drinker line components or decreases in water consumption. If using drinking water acidification treatments, both water pH and broiler water consumption should be monitored. Alternatively, other commercially available feed withdrawal water treatments include chlorine dioxide or a combination of chlorine dioxide and acidification. Hydrogen peroxide and cetylpyridinium chloride treatments are currently under investigation. Time off feed at the farm, time on water prior to catching, time required to catch, transport time, and lairage time all need to be scheduled accurately in order to achieve the recommended 8-12 hours of feed withdrawal prior to slaughter.

		Optimal Feed Withdrawal
Carcass	Neck	Crop should not be visible
	Abdomen	Should be concave
Alimentary Tract	Crop	Should not contain feed
	Jejunum	Should be flat

Catching of Broilers for Loading

Around the world there are several types of catching used. Broilers can be caught by the back, legs, or mechanically. Each method has advantages and disadvantages regarding animal welfare, catching time, and carcass quality after slaughter.

In a study comparing broiler catching by either a 1-leg or a 2-leg method, no significant differences were observed in wing, leg, or body lesions (Langkabel et al. 2015). However, the authors noted that the broilers that were caught by the 2-leg method exhibited increased “unrest” as compared to the broilers caught by the 1-leg method. In addition to a possible negative effect of 2-leg catching on the broilers, the catching crew experienced greater difficulty in catching broilers by both legs. Workers had to spend additional time crouched to gather both legs, leading to greater physical stress.

Mechanical catching has been shown to be as good as or better than hand catching of broilers (Lacy and Czarick 1998; Schilling et al. 2008). However, large scale implementation of mechanical catching in the United States has not been practical due to the variability in broiler houses. Factors such as interior barn height and building supports can hinder the ability of equipment to maneuver in the house. Additionally, broiler houses in the United States are often build on dirt floors which can lead to floor variations making it more difficult for the mechanical equipment to safely catch all broilers.

Catching Crew Training

Whatever the method of catching, training of catching crews is vital for optimizing animal welfare. A positive catching crew employee attitude which is influenced by the attitude of management, employee supervision, and crew training have all been demonstrated to significantly improve broiler welfare (Kettlewell and Turner 1985; Grandin 1994; Ekstrand 1998). Individuals on catching crews can compromise the welfare of broilers for several reasons.

The employee may not know how to properly catch, carry, and load the broilers.
The employee may know how they are supposed to catch, carry, and load but not yet be skilled at doing it properly.
The employee may not be physically capable of proper catching, carrying, and/or loading.
The employee may not be concerned with broiler welfare.

In a study investigating catching team training, mistakes including catching by means other than the back, sudden movement, crowding of birds, startled birds, too many broilers per crate, house curtain position, and loading speed were recorded and compared by catching team age, weeks of training, and time of day against the frequency of back scratches at the processing plant (Pilecco et al 2013). Younger workers required more training time and tended to catch more quickly resulting in startled broilers. Catching during a daylight shift also increased the observed rate of back scratches. However, by the end of four weeks of training, all crews had achieved lower rates of broiler back scratches.

Sick, severely injured, and dead broilers should not be loaded into crates for transport to the processing plant. However, frequently, every live broiler is loaded regardless of condition. In some broiler systems, the farmer is paid based on truck weight entering the live haul area of the processing plant. Incentives to minimize the loading of sick or injured birds should be implemented to encourage culling of these birds instead of loading. Catching crew incentive programs are currently implemented adjusting pay based on the occurrence of DOAs. For example, a crew presenting with 0.001% DOAs will receive a higher base pay per 1000 broilers loaded than a crew presenting with 0.002% DOAs.

Recording observations during catching can be useful for broiler house, working condition, and catching crew evaluations. Keeping records can help to ensure the humane treatment of broilers and proper training of catching crews which will enhance profits downstream in the processing plant.

Transportation to the Processing Plant and Lairage/Live Haul:

Once the broilers have been loaded into crates and onto trucks, the transport and lairage environment must be considered. When ambient temperature is high, additional measures such as keeping the broilers under shade and cooling with fans and misted water can be utilized during holding at the farm and during lairage to minimize overheating. When ambient temperature is low, broilers need to be protected from wind, particularly in cold and wet conditions. The time during transportation and lairage impact both individual bird welfare and the frequency of DOAs. During transportation to the processing plant and after lairage, significant increases were found in splayed legs, supine broilers, and stuck wings (Jacobs et al 2017). Frequency of DOAs was found to significantly increase with both longer transport times and longer lairage times

(Nijdam et al. 2004). While the locations of the farms and processing plants cannot be changed, traffic patterns should be considered when scheduling broiler catching to minimize transport time. Optimizing scheduling for broiler arrival in lairage may also help with minimizing the amount of time the broilers are held prior to slaughter.

Lighting	Catching in low light minimizes stress
Noise	When necessary speak quietly and minimize loud noises (e.g. slamming doors, moving crates, fork-lift noise, etc.).
Movement	Walk slowly around the broilers and avoid fast movements.
Piling/Suffocation	Load broilers from the end of the barn while slowly moving into the barn as broilers are being caught. Check corners to ensure broilers are not piling.
Humane Handling	1. Do not carry broilers by the wings or neck. Carrying in a group by 1 leg is recommended over 2 legs. 2. Carry 2-4 broilers per hand or as instructed by supervisor 3. Do not swing, throw, or drop broilers. 4. Do not pass broilers between people. 5. Place broilers gently in crates. 6. Ensure broilers are in an upright position.
Crate Distance	Do not carry broilers more than 5 meters before placing in crates.
Crate Density	Density will depend on crate size, broiler size, and environmental conditions. In hot conditions, crate density should be decreased. In cold conditions, crate density may be able to be increased. Catching crews should be advised on how many broilers should be loaded into each crate for each house to be caught.
Crate Condition	Broiler crates must be in good condition. Broken or damaged crates can cause broiler injury or allow broilers to escape during holding or transport.

Heat Stress During Loading, Transport, and Holding

Acute heat stress can occur during loading, transport, and holding of broilers at the processing plant, particularly in geographical regions with high summer temperatures. This heat stress can lead to DOAs as well as pale meat with lower water-holding capacity and greater cook loss. In order to improve bird welfare and product quality, multiple approaches can be used to keep the birds cool during this time.

Appropriate feed withdrawal to minimize heat production
Avoid unnecessary bird movement during catching
Avoid crowding of birds in the house during catching
Decrease the number of birds per coop
Park transport trailer in the shade during loading at the farm
Utilize fine misters and fan systems
Choose a route to the processing plant to minimize potential stops (e.g. traffic lights)
Hold birds at the plant in a shaded area with adequate ventilation, misters, and fans

Conclusion

Consideration of the most appropriate methods for your operation in harvesting market age broilers can have a significant impact on yield and carcass quality in the processing plant. When feed withdrawal, catching, loading, transportation, and lairage are well managed, returns can be maximized through improved yields and products.

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O[25] Effects of Pre Slaughter Feed and Water Withdrawal Durations on Electric Resistances, Breast Meat Quality Characteristics and Carcass Defects of Broiler Chickens

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Abstract

Removal of feed from slaughter-aged broilers is a standard practice. Before broiler chickens are transferred to slaughterhouse, feed is withdrawn on-poultry house to enable in emptying the digestive tracts of the birds and reduce the risk that the carcasses will be contaminated during processing. Also, chickens are without water in this period when they move to the slaughterhouse. Considering animal welfare, it is recommended that without feed period should not exceed 12 hours prior to slaughter. The purpose of this study was to evaluate the effects of feed and water withdrawal periods (4, 8, 12 h) on body electric resistances (Ω), some breast meat quality characteristics and carcass defects of broiler chickens. The study was conducted with a total of 3 experimental groups with an average weight of 2386±404g, each with 30 chickens (15♀:15♂) at 39 days of age. In the experiment, feed and water were withdrawn 4, 8 and 12 h before slaughter. And then the chickens were slaughtered by the electrical stunning. The electrical resistances of chickens were calculated by using the voltage (V) values determined during electrical application. The amount of blood loss of chickens was measured during the slaughter. Defects in the shape of hemorrhages and bone fractures were detected in chicken carcasses. In order to examine breast meat quality, pH, color and drip loss values were measured. When the duration of without feed and water was extended, it was determined that blood loss increased in chickens but body electrical resistance, redness in breast meat, hemorrhages in wings and legs decreased. Research findings revealed that the 12 hours fasting and thirst duration compared to the others increased the amount of blood loss in broilers, and decreased carcass defects.

Keywords: Fasting, electrical resistance, meat quality, carcass defect

O[26] The Effects of Organic Acid Supplementation by Drinking Water on Performance, Foot Pad Dermatitis Incidence and Carcass Yield in Broilers Under Field Conditions

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Abstract

This study was performed to investigate the effects of organic acid supplementation by drinking water on performance, foot pad dermatitis and carcass yield in broilers under field conditions. This experiment was conducted with a total of 115.700 broilers at four houses belonged to two broiler farms located in Bursa and Balıkesir. Each of two houses at farms was randomly selected as “control house”, and the other house was “treatment house”. In the control houses, standard management and nutrition practices were applied, whereas an organic acid combination (formic acid, lactic acid, propionic acid and acetic acid) was supplemented into drinking water in the treatment houses. The supplementation of organic acid (1 lt per tonnes water) was started at 11 days of age in first farm and at 7 days of age in the second farm. In the study, data for final live weight, feed conversion rate, total mortality and European efficiency productivity factor (EPEF), incidence of foot pad dermatitis and carcass yield were measured and calculated. At the end of the experiment (at 40 days of age), final live weight was 2778 g in the treatment house and 2654 g in the control house in the first farm, 2622 g in the experiment house and 2452 g in the control house in the second farm. Total mortality was found to be significantly different in the first farm. It was %5.05 in the experiment house and %6.6 in the control house. EPEF was higher in the treatment houses (357.2 and 391.7 respectively) than the control houses (339.9 and 384.0 respectively) at both of farms. The supplementation of organic acid had a detractive effect for foot pad dermatitis incidence at slaughter age. In both of experiments, incidence of foot pad dermatitis was 18% and 15% in the control houses, whereas 13% and 9% in the treatment houses. When considering the findings, it can be concluded that organic acid supplementation into drinking water had a positive effect on production parameters in broilers under field conditions.

Key words: broiler, organic acid, feed conversion rate, foot pad dermatitis

IS[14] How to Improve Fertility and Hatchability in Broiler Breeders?

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Summary

Broiler breeders are the parent stock of broiler chickens and therefore responsible for millions tons of poultry meat. Appropriate management in the rearing and laying period is essential to produce a high number of fertile eggs and high quality broiler chickens. There is a wide range of factors influencing broiler breeder production parameters, health and economics of a flock. A clear focus on the basic management factors (housing, breed, climate, nutrition, bio security, etc.) can limit negative production responses in an early stage, improving overall profitability of the production chain. A key issue in broiler breeder production is the decrease in fertility and hatchability of eggs, especially in the second part of the laying period. This decrease in fertility may be caused by a wide range of factors such as mating behavior, development of percentage of males during the entire laying period, spiking male's protocol, development of body weight of males and females, feather cover, housing system and nutrition. In general, optimizing the above mentioned management factors during the laying period will lead to maximizing reproduction performance of broiler breeders. In more detail, it is important to improve mating behavior, implement different male management strategies, apply spiking males, control bodyweight of males and females, improve feather cover, use modern housing systems, and feed the females low CP diets during the second part of lay.

Introduction

Poultry meat is one of the most important protein sources in human diet and production is worldwide growing. Global poultry meat production in 2000 was 69 million tons and this increased to over 97 million tons in 2010 (Windhorst, 2011). This equates to an annual production of approximately 70 billion broilers originating from approximately 600 million broiler breeders. So a relatively small number of broiler breeders has a major impact on the poultry meat chain and optimizing management of breeders will have benefits for the total chain. Broiler breeders need to produce first class and healthy chicks (Zuidhof et al., 2007). Due to the continuing increase in the genetic potential of the offspring (e.g., Havenstein et al., 2003a,b; Renema et al., 2007; Zuidhof et al., 2014) this is becoming increasingly challenging. A key issue in broiler breeder production is the decrease in fertility and hatchability of eggs, especially in the second part of the laying period. Fertility of hatching eggs declined from 88.8% in 2000 to 84.7% in 2005 (van Emous, 2010). This decrease in fertility may be caused by a wide range of factors such as strain, health status of the flock, egg size, egg weight, egg quality, egg storage duration and conditions, egg sanitation, season of the year, and age of the breeders (as reviewed by Yassin et al., 2008). Besides these factors, mating behavior, development of percentage of males during the entire laying period, spiking male's protocol, development of body weight of males and females, feather cover, housing system, and nutrition play a very important role on fertility and hatchability. Therefore the overall objective of the present presentation is to give an overview of the effects of different management interventions during the laying period on fertility and hatchability.

Mating Behavior

Because males and females under natural conditions have separate social hierarchies, and males dominate females passively, aggressive behavior to hens by mature males is uncommon (Wood-Gush, 1958; Kruijt, 1964; Rushen, 1983). In contrast, in commercial housing it has been shown that male broiler breeders may demonstrate high levels of aggression towards females, mainly during the performance of mating behavior, whereas courtship behavior was virtually absent before mating (e.g. Jones and Prescott, 2000; Millman et al., 2000a,b; Duncan, 2009; de Jong et al., 2009). This is possible one of the major reasons why females tend to remain on the slatted area or hide in the nests instead of being in the litter area (where the majority of males are present) at the end of the light period. Birds showed often severe wounds on the back, the back of the head and along the torso beneath the wings which was related to the aggressive mating behavior (Millman et al., 2000a). Sexual behavior of male broiler breeders has been described as rough, the males pecking or chasing females and forcing copulations (Jones and Prescott, 2000; Millman et al., 2000a; Jones et al., 2001). This phenomenon of forced copulations is also reported in another study (Leone and Estevez, 2008). They found that females stayed on the slatted area instead of the litter area, and found injuries in female broiler breeders during lay. However, they suggested that the cause of the rough mating behavior may be that males reach sexual maturity at a younger age as females. It is highly plausible that both aggressive or rough mating behavior and too early maturation of males play an important role in injuries, fear and stress of the females. In a field study on eight Dutch broiler breeder farms the behavior of males as well as females had been studied in more detail. It was concluded that male behavior towards females could be described as 'rough', but also female courtship behavior appeared to be incomplete. It was remarkable that females in general did not show crouching behavior in response to male approach. Females often showed struggling behavior during mating or tried to escape from the male. This incomplete mating behavior of the males and females may explain the low percentage succeeded matings (44% between 20-28 weeks of age) and why roughly 90% of the matings was forced (de Jong et al., 2009). They also found that courtship behavior was almost absent before mating, confirming earlier experiments (Millman et al., 2000a; Jones et al., 2001). Because females do not recognize male signals the behavior of the females may thus seem to be incomplete. In the opposite, it has recently been shown in different female broiler breeder lines that female behavior evoked aggressive male behavior (Moyle et al., 2010). Feed restriction did not play a role in the rough mating behavior (Millman and Duncan, 2000) but genetics may have an effect. It was suggested that factors like separate rearing of males and females, large group sizes and high stocking density may also play a role by the development and recognition of courtship behavior and proper responses to signals of the other sex (de Jong et al., 2009).

It has been estimated that mating frequency is 5 to 10 times higher in a broiler breeder house as compared to flocks of chickens housed under natural conditions (Van Emous, 2010). This relatively high frequency of mating may affect the relationship between the males and females, resulting in females avoiding males as hypothesised by Fontana et al. (1992).

Percentage of Males

Mating behavior of both males and females is important with respect to maximizing the production of fertile eggs. However, broiler breeder males may show rough, aggressive behavior towards females during mating. This rough male behavior may lead to feather- and skin damage and fearfulness in females. As a result, females may hide in the nests and fertility will be negatively affected (Millman, et al., 2000). One of the reasons for male aggression may be that males reach maturity earlier than females, leading to forced copulations and distress in the females, which in turn results in females hiding on the slats and in the nests (Leone, et al., 2007). However, also with proper management in relation to sexual development of males and females, rough and aggressive mating behavior of the males has been observed. In addition, it was observed that courtship behavior in males was virtually absent (de Jong, et al., 2009; Hocking and Bernard, 2000; Jones and Prescott, 2000; Millman, et al., 2000). It has also been observed that females do not seem to respond properly (with crouching behavior) to male approach (de Jong et al., 2009).

It is unknown why male broiler breeders show this rough behavior towards females during mating. Rough mating behavior was not related to aggressive behavior per se (Millman and Duncan, 2000b) and feed restriction in rearing was not related to rough mating behavior (Millman and Duncan, 2000a). Genetic background may play a role, as males from laying strains responded less aggressive during mating than broiler breeder males (Millman and Duncan, 2000b). Reducing the stocking density, thus, providing males with more space to perform courtship behavior, indeed had a positive effect on the quality of the mating behavior (more courtship behavior and more successful matings) and also resulted in a higher egg production, more fertile eggs and a higher number of chicks per hen (de Jong et al., 2011). Jones et al. (2001) showed that enriching the light conditions with UVA improved the transmission of sexual signals and thus the quality of the mating behavior.

Over-mating might be avoided by separating females and males temporarily during the day. Based on this hypothesis, a new housing system for broiler breeders, called the Quality Time® house (QT), has been developed (van Emous, 2010). Males are separated from females during 5 hours per day, using a separate feeding system and a moving fence. After a successful pilot experiment, two on-farm experiments were carried out in a broiler breeder house with 15,000 birds. The house was divided in six compartments. In the QT compartments more voluntary and successful matings were observed. Also, quality of the sexual behavior improved which resulted in an improved feather cover between 37 and 48 weeks of age in the QT compartments as compared to the control compartments. Separating males from females did not increase aggressive behavior between the males in the male pen (van Emous, 2010).

Spiking Males

The percentage of males at the start of the production period is between 8-11% and this decreases due to selection and mortality of males. At the start of production around 23 weeks of age 7.5-9% males are present in a flock (EFSA, 2010). Reasons for selection of males are absence of mating activity or health problems (e.g. leg problems). About 15-25% of the males is selected during the production period. In some countries ‘spiking’ of males is common practice. Inactive males are removed from the flock and replaced by younger and more active males to maintain the production of fertile eggs at a high level (Leeson and Summers, 2000). However, spiking involves the risk of introduction of pathogens and it may be stressful to the birds because male aggression may increase (EFSA, 2010). Sometimes intra-spiking (swapping older males at the same farm from one house to the next) is used as a method to increase male activity and thus fertility, which has a much lower biosecurity risk (Casanovas, 2000).

Body Weight Control of Males and Females

During the last 4 to 5 decades, increased selection for fast growth and breast meat had result in fertility and hatchability problems in broiler breeders after 50 weeks of age (Kirk et al., 1980). The drop in sexual activity is associated with the fact that males gaining too much body weight while aging. Sexual behavior and fertility at 28, 38 and 58 weeks of age was investigated by Duncan et al. (1990). They feed the males different energy levels to obtain different target body weights. They observed a decrease of all sexual behavior, including courtship and copulations, which suggested a decline in sexual activity. They concluded that the reduction in fertility was caused by their body composition or more precise the huge amount of breast meat which negatively affected the possibility for cloacal contact. In a comparable study, Wilson et al. (1979) studied the relationship between body weight and several other physical characteristics in broiler breeders for both natural matings and artificial insemination. They found for natural matings, a tendency for a negative relationship between fertility and body weight, breast angle and hip width. The authors speculated that the combination of broad breast and wide hips might lead to difficulty in mounting or difficulty with balance, or lead to a higher incidence of leg weakness, all of which might interfere with successful mating.

In the past, obesity, mainly in the second phase of the laying period, was a major problem in broiler breeder flocks and resulted often in a decreased reproduction rate during the laying period (Bornstein et al., 1984; Leclercq et al., 1985; Cahanar et al., 1986; Robinson et al., 1993). Overweight hens have sperm storage problems (due to the fat deposition in the sperm storage glands) and physical problems during the cloacal contact during natural mating (Mc Daniel et al., 1981). The body composition of breeders, however, has changed dramatically during the last five to six decades (Havenstein et al., 2003a; De Beer, 2009). In modern broiler breeders, obesity is not an issue anymore, due to the selection of strains with increased breast muscle and decreased fat pad deposition characteristics (Havenstein et al., 2003a). The selection for increased feed efficiency, growth rate and body fat content has not only affected the offspring but also the parent stock. This was recently confirmed by Eitan et al. (2014) who compared a 1980 to a 2000 breeder strain. The 2000 strain contained 42% more breast meat (21.2 vs. 14.9% of BW) and 50% smaller abdominal fat pad (2.7 vs. 5.4% of BW) compared to the 1980 strain.

It is suggested by Sun and Coon (2005) that feeding a higher fat diet during the laying period result in more body weight gain, larger eggs and more carcass fat in female broiler breeders. It is, therefore, suggested by some researchers (Sun and Coon, 2005; de Beer, 2009; Decuypere et al., 2010) that a certain proportion of body fat in breeders at the onset of lay is necessary for maximum egg production.

This was confirmed by a study of van Emous (2015) that modern broiler breeders have a lack in fat reserves (and thus body weight) during the entire laying period.

An inventory in practice of van Emous (non-published data) already showed that females with BW above 3.9 kg at the end of the laying period produced eggs with the highest hatchability. For males it is the opposite, BW of males must be lower than 5 kg at the end the laying period for maximum hatchability.

Feather Cover

The quality of feather cover of broiler breeders has decreased over the last decade and an impaired feather cover decreased welfare of the broiler breeders (van Emous, 2010). Feathers play an important role to protect broiler breeders for skin damage caused by sharp objects in the house and for damage during rough mating behavior of the male (de Jong et al., 2009). Besides the important role as protection for the female, feathers are very important for thermoregulation of the birds. Birds are losing heat by the absence of the insulation layer of feathers. In a study with laying hens (Peguri and Coon, 1993) was found that 50% bald hens need 9% more feed through an increased energy requirement for maintenance. The third major issue in feather cover is the function in preventing feather damage. A good feather cover can also help as grip for the male during mating behavior and is furthermore a good indicator for the health status of the birds (Van Krimpen, personal communication). The cause for this poor plumage condition is not yet clear. Nevertheless, a farm inventory of van Emous (non-published data) showed that factors such as feeding space and behavior of males and females during feeding time seems to be highly relevant. Furthermore, in that inventory a breed effect was found on the quality of feather cover of broiler breeders. In an on farm study van Emous (non-published data) found that a worst feather cover in the beginning of the laying period (30 wk of age) negatively affected the hatchability of the total laying period. In the literature, only a few studies have been conducted to the effects of dietary energy and protein on plumage condition. A study with broiler showed that a dietary protein content >16% should be sufficient at an early age to ensure plumage development (Twining et al., 1976). It is questionable whether the results of this dated study still apply for the modern broiler breeder. A low protein diet during the rearing period, however, had a negative effect on feather cover quality (van Emous et al., 2014; van Emous et al., 2015). In the first experiment (van Emous et al., 2014), feather cover was inferior on the low protein diet at 6 and 11 wk of age while this difference disappeared from 16 wk of age onward. In the second experiment (van Emous et al., 2015), feather cover was inferior on the low protein diet during the entire rearing period. It is, therefore, suggested that the protein and amino acid levels of the diets in the studies here were critical or deficient, in particular those amino acids needed for feather growth and development. The effect of daily protein intake on feather growth in broilers was previously reported by Twining et al. (1976), Aktara et al. (1996), Melo et al. (1999), and Urdaneta-Rincon and Leeson (2004). Data of van Emous et al. (2014) were used to analyse the linear relationships between the total crude protein intake at different phases during the rearing period on feather cover score. They concluded that the effect of a low total CP intake on feather cover score was much more pronounced between 2 and 6 wk of age than between 6 and 15 wk of age. It is, therefore, concluded that total CP (and AA) is a critical factor in development of feathers cover during rearing till approximately 6 wk of age.

Housing System

The transition from the rearing period to the production period involves transportation to the production house, usually at a separate production farm. The production period starts between 18-22 weeks and lasts until 60-65 weeks, dependent on the performance of a breeder flock. Males and females are reared separately and mixed at the beginning of the production period, and in Europe usually arrive on the same day at the production farm (EFSA, 2010; Van Tuijl, Aviagen, 2016, pers. comm.). The majority of the breeders are housed in floor house systems during the production period. Houses may be artificially lit, with or without windows or open-sided with curtains, which is dependent on legislation, region and climate. Artificial light in addition to natural light can be used to stimulate reproduction. Although less common, broiler breeders can also be housed in multi-tier colony cages during the production period. These are furnished cages with laying nests, perches and also a small litter area (de Jong and Swalander, 2013). Aviaries are not used for broiler breeders. Broiler breeders in production can also be housed in conventional cages where artificial insemination is applied which is uncommon in Europe but may be found in other parts of the world (EFSA, 2010). Floor house systems consist of a litter area and a certain proportion of slatted floors from which the nests can be accessed. The proportion of littered floor versus raised slatted floor (plastic or wooden slats) may differ between countries and regions, and the lay-out is also regionally dependent.

Housing systems can be divided roughly in two different layouts: breeder houses with a central litter floor or houses with a central slatted floor. In general, 100% of the breeder houses in Europe is equipped with a central slatted floor with an automatic community nest in the middle of the slatted floor. In the rest of the world the majority (approx. 90%) is equipped with a central litter floor with manual or automatic nests on both slatted floors at the side walls. In a field experiment of Leone and Estevez (2008), vertical panels (of 70 x 70 cm) were placed in a staggered pattern every 4.5 m in the central litter area. Access to cover panels improved egg production by 2.1% and maintained better hatchability and fertility throughout the breeding cycle leading to an additional 4.5 chicks/female. Providing enrichment in the form of cover panels improved reproductive performance, most likely by increasing males mating opportunities and reducing female stress. These panels attracted females to the litter floor, thereby decreasing the competition for females.

In Europe very good reproduction performance (high egg production, high hatchability and low floor eggs %) with breeder houses with a central slatted floor layout are recorded (van Emous, personal observation). It is postulated that this layout is more in line with the natural mating behavior of the breeders. During behavior observations it was observed that roughly 90% of the matings are found place on the litter arear (van Emous, non-published data). This means that the litter area is the most important compartment of the breeder house. This is being strengthened by the separated male feeders located to the walls of the breeder houses. It is suggested that in central slatted floor houses, mixing of females and males, especially with a short day-length (max. 14 h), good litter quality and frequent visits of the farmer in the house is beneficial for fertility and hatchability. Moreover, a field test on both breeder houses lay-outs will start in the summer of 2019 to investigate the differences in egg production fertility/hatchability and mating behavior.

Nutrition Females

A negative effect of a high daily crude protein intake (> 25 g/d) during the laying period on fertility or hatchability of eggs has been reported by Pearson and Herron (1982), Whitehead et al. (1985) and Lopez and Leeson (1995a). A decreased hatchability of fertile eggs could be explained by an increased embryonic mortality as shown by Pearson and Herron (1982) and Whitehead et al. (1985). Ekmay et al. (2013) showed that increasing levels of dietary lysine and isoleucine at peak production results in a reduction in fertility. An explanation for this effect on fertility was postulated by de Beer (2009), who suggested that an increase in CP intake leads to an increase in nitrogen excretion (de Beer, 2009; Lopez and Leeson, 1995a). This excessive nitrogen excretion may lead to an alkaline environment near the cloaca, where the sperm host tubules are located, with detrimental effects on the semen quality stored in these tubules.

Feeding high yield breeders high levels of amino acids (e.g. lysine) will also lead to more muscle production and this extra muscle requires more energy to maintain (de Beer, 2009). Therefore, during the last decade several researchers have reported that broiler breeders need a certain proportion of body fat at the onset of lay for subsequent reproductive performance (Sun and Coon, 2005; de Beer, 2009; Mba et al., 2010). Because tissue growth is directly affected by dietary nutrient composition, a nutritional approach to this topic was highly relevant.

The results from van Emous et al. (2015) showed conclusively that feeding birds a high energy diet (less daily protein intake) during the second phase of lay improved hatchability of fertilized eggs. These results are in agreement with Pearson and Herron (1982), Whitehead et al. (1985) and Lopez and Leeson (1995a). The differences in hatchability of the fertile eggs were caused by differences in embryonic mortality. A higher or lower embryonic mortality leads to a lower or higher hatchability of fertile eggs, respectively. This observation supports the earlier work of Pearson and Herron (1982) who found that lowering daily protein intake (27.0 vs. 21.3 g/bird) resulted in a decreased mortality and malformation of embryos. The decreased embryonic mortality in birds fed the high energy diet (low daily protein intake) in the study of van Emous et al. (2015) can be explained by the lower egg weight (68.7 vs. 69.1 g). Larger eggs have a higher eggshell conductance (EC) due to an increased pore density or pore size (Shafey, 2002). A higher EC increased vital gas exchange and water loss which causes, respectively, an increased early and late embryonic mortality (Peebles et al., 1987).

The effect of an improved hatchability and decreased embryonic mortality, while feeding the high energy diet (low daily protein intake), was underlined by the decreased proportion of second grade chicks in the study of van Emous et al. (2015). The relationship between low embryonic mortality and less second grade chicks was previously found in studies of Reijrink et al. (2010) and Molenaar et al. (2011).

Conclusion

In general, optimizing the above mentioned management factors during the laying period will lead to maximizing reproduction performance of broiler breeders. In more detail, it is important to improve mating behavior, implement different male management strategies, apply spiking males, control BW of males and females, improve feather cover, use modern housing systems, and feed the females lower CP diets during the second part of lay.

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O[27] The Influence of Dietary n-3 Fatty Acid and Rooibos (*Aspalathus linearis*) on Semen Quality, Sperm Fatty Acids, and Reproductive Performance of Aged Ross Breeder Roosters

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Abstract

Rooibos (*Aspalathus linearis*) an indigenous plant to South Africa being especially popular for its antioxidant and therapeutical features, has been used in the present study to evaluate the effects of dietary fish oil (FO) and fermented rooibos on semen quality, fatty acid (FA) composition, and reproductive performance of aged roosters. Seventy-two 52-wk-old Ross broiler breeder roosters were randomly assigned to a 2 × 3 factorial arrangement in a completely randomized design with two FO levels (0 and 2 %) and 3 rooibos levels (0, 1.5 and 3 %) for 13 wks. During the trial birds' semen parameters and lipid peroxidation were evaluated every 2 wks but in the current manuscript only average results of whole period are reported. Fertility and hatchability were determined using 120 artificial inseminated hens at the end of the experiment (wk 64).

Results showed that dietary FO significantly decreased linoleic, arachidonic, ΣMUFA, n-6 and n-6/n-3 ($P < 0.05$) in roosters' sperm. Semen concentration, semen volume, and sperm livability significantly ($P < 0.05$) increased by dietary rooibos. There was an interaction between FO and rooibos ($P < 0.05$) where inclusion of dietary rooibos (1.5 and 3 %) significantly increased ($P < 0.05$) sperm livability so that birds fed on diet containing rooibos had the highest level of live sperm compared to birds fed on control or only FO diet. FO × rooibos interaction was also significant for sperm motility ($P < 0.05$). Increasing level of dietary rooibos from 1.5 to 3% significantly increased sperm motility when 2% FO was included in the diet ($P < 0.05$). In comparison to nonsupplemented diet with rooibos, lower concentration of MDA was recorded in birds received 1.5 and 3 % rooibos ($P < 0.05$). Higher significant percentage of fertility rate ($P < 0.05$) was observed in FO group that was supplemented with 3 % rooibos compared to other groups. Moreover, FO and 3 % rooibos as main effects significantly increased absolute and relative testes weight ($P < 0.05$), however, the FO × rooibos interaction was not significant for these parameters ($P > 0.05$). Birds' body weight was not affected neihter by dietary FO and rooibose or their interaction. In conclusion, supplementation of aged roosters' diet with FO (in terms of n-6:n-3) and 3% rooibos (as an antioxidant) can be a helpful nutritional strategy in attenuating age-related subfertility.

Introduction

Reproduction plays an important role in poultry industry. Fertility rate in a breeder stock depends on both male and female birds while the contribution of male can be more significant when females' number exceeds that of males (Scott, 1973). On the other hand, while, the lipid content of the plasma membranes of spermatozoa bears a functional role in sperm's fertilizing capability (Cerolini et al., 1997), due to high PUFA content of sperms, spermatozoa are extremely susceptible to lipid peroxidation in reaction to oxygen species, which is closely associated with male infertility (Surai et al., 2001). In spite of this, many studies have shown that dietary supplementation of PUFAs, especially omega-3 (n-3) PUFAs, significantly increases sperm fertility in birds (Safari Asl et al., 2018; Zanini et al., 2003; Kelso et al., 1997).

Polyphenols is characterized by the presence of several groups involved in phenolic structures such as flavonoids and phenolic acid (Al-Gubory et al., 2010) *Aspalathus linearis*, commonly known as rooibos, naturally grows in Cederberg area, the western parts of Western Cape Province in South Africa and is commercially used for herbal tea or tisane (Dahlgren, 1968; Mckay and Blumberg, 2007). Based on its antioxidant polyphenols activities, *Aspalathus linearis* was shown to possess anticarcinogenic and antimutagenic properties (Marnewick et al., 2000). So, unfermented and fermented rooibos contain high levels of antioxidants which inhibit reactive oxygen species (Monsees and Opuwari, 2013). Additionally, rooibos includes hepatoprotective effect, phytoestrogenic activity, antispasmodic effects, antihaemolytic effect, anti-aging properties, anti-microbial and antiviral effects, vasodilatory effects, and modulation of lipid profile amongst others, which can be counted as its health beneficiary effects (Joubert et al., 2008; Marnewick et al., 2009; 2011).

The aim of the current study was to evaluate the possible protective effects of fermented rooibos on semen quality and quantity, sperm FAs profile, testes weight, and semen malondialdehyde as an indicative of oxidation level in aged Ross 308 breeder roosters fed on diets with or without dietary fish oil as n-3 source (0 and 2 %) and fermented rooibos (0, 1.5 and 3 %) as a natural antioxidant.

Materials and Methods

The chemically active constituent of rooibos tea (Stokkies tea® Biedouw valley; South Africa; total polyphenol and major flavonoid content) was determined based on the method described by Beelders et al. (2012). The product which is a mixture of mainly fermented and a small amount of unfermented rooibos consists of both woody parts and leaves of the plant. The bushes can be found in its original habitat called Biedouw valley.

Totally seventy two 47-wk-old broiler breeder roosters (Ross 308) with similar mean of weight were housed in individual cages equipped with inside nipple drinkers and an outside galvanized feeder under uniform environmental conditions (15L: 9D light time table at 21 to 23 °C). Birds were randomly allotted to six treatments of similar mean ± SD weight each of which included 12 male broiler breeders (6 respective replicates of 12 birds in each).

Three levels of rooibos (0, 15, and 30 g of rooibos per kg of diet) and fish oil (FO; 0 % and 2 %, respectively) were tested in a completely randomized design with a 2×3 factorial arrangement of treatments. Birds were categorized into 6 groups and fed based on the following treatments:

F0R0= control diet with no rooibos and/or fish oil; F0R1.5= containing only 1.5 % rooibos; F0R3= containing only 3 % rooibos; F2R0= containing only 2 % fish oil; F2R1.5 containing 2 % fish oil and 1.5 % rooibos; F2R3 containing 2 % fish oil and 3 % rooibos.

The control and experimental diets in all trial groups were a standard commercial mash for Ross broiler breeder roosters (data has not shown). The experimental diets in which bentonite was replaced by the rooibos were formulated to meet nutrient requirements of the broiler breeder roosters, as established by the Ross 308 parent stock nutrition specification (2016). The FA profile of FO and rations were also determined (data has not shown). Individual live body weight of each bird was recorded weekly from 48 to 64 weeks of age. Feed consumption was measured weekly. Roosters were habituated (for almost 3 wks) by abdominal massage for semen collection (48 to 50 week of age; Burrows and Quinn, 1937). Semen was collected every 2 wks between 52 and 64 weeks of age. The ejaculates from the birds in each replicate (n = 3) were pooled and evaluated as a single sample for semen volume, sperm concentration, sperm viability, abnormal sperm, and sperm motility (Schafer and Holzmann, 2000; Golzar Adabi et al., 2007; Golzar Adabi et al., 2011).

At the end of the experiment, three birds from each pen were euthanized by cervical dislocation. The abdominal cavity was opened and testes dissected out. The weight of each testis was measured by using an electronic analytical scale. Every two wks interval from 50 to 64 wks of age, the pooled semen samples of three birds in each replicate were used for determination of lipid peroxidation of the semen. The concentration of malondialdehyde (MDA), an index of lipid peroxidation and oxidative stress, was determined with thiobarbituric acid (TBA) method as described by Botsoglou et al. (1994).

Artificial insemination was performed at the end of experiment according to the method of Akhlaghi et al. (2014a) with some modifications. Pooled semen obtained from 12 roosters in each treatment was then diluted in buffer. At 14:00 pm on certain days, 20 hens belong to each experimental groups inseminated by pooled semen samples (120 hens total). The hens were inseminated (200×10^6 sperm/hen) for two times per week. The eggs were collected up to 5 d after the last AI and then were numbered and stored at 13°C and 75 % humidity until incubated for fertility assessment and hatchability rates. In each group, 300 settable eggs were set (60 eggs in each weekly set) on incubator trays and disinfected with formaldehyde gas for 15 min. According to recommended concentration, 1.2 mL of formalin was added to 0.6 g of potassium permanganate. Hatching rate was calculated after 21 days of incubation based on the number of fertilized eggs.

Statistical analysis

All percentage data were subjected to arcsin square root transformation (Steel and Torrie, 1960). All data are presented as least squares means ± SEM. Single and repeated measurement data were analyzed by PROC GLM and PROC MIXED, respectively (SAS Institute, 2001). When necessary mean separation was accomplished by using Tukey's post hoc test and a probability value of less than 0.05 was considered significant, unless otherwise noted. The statistical model was:

$$y_{ijk} = \mu + a_i + b_j + (a \times b)_{ij} + e_{ijk}$$

In the model, y_{ijk} was any observation; μ was the overall mean; a_i was the effect of the FO (control, inoculated); b_j was the effect of the addition of the rooibos (control, inoculated); $(a\times b)_{ij}$ was the interactions; and e_{ijk} was error term.

Results

Mean initial and ultimate body weight (BW) of the birds in different treatment groups showed no significant differences. There was no interaction of FO and rooibos on body weight. FA composition of the diets (data has not shown) clearly reflected the origin of the added FO. As expected, the FO diet contained the lowest ratio of n-6/n-3, for example the ratio between n-6 and n-3 FAs were 16.12 % and 3.11 % in the basal and FO oil diet, respectively. Fatty acid composition of spermatozoa elucidates the effects of varying the FA profile of the feed on the composition of sperm lipids (data has not shown).

Data of semen parameters, fertility, hatchability, BW, absolute and relative testes weight are summarized in table 1. The FO × rooibos interaction was found to have significant effect on sperm motility, sperm livability and MDA ($P < 0.05$) but not on sperm concentration, semen volume and abnormal sperm ($P > 0.05$). On the other hand, inclusion of rooibos at 1.5 and 3 % level ($P < 0.05$) significantly improved sperm concentration. Increasing FO level, as the main factor, from 0 to 2 % significantly boosted the semen MDA level ($P < 0.05$), while inclusion of dietary rooibos had a remarkably diverse effect and decreased ($P < 0.05$) the semen MDA level. A significant interaction between the FO and rooibos ($P < 0.05$) was also observed for MDA. The results also showed that, MDA level significantly decreased in diet containing both levels of rooibos with or without FO. With dietary FO as main factor and its interaction with rooibos no significant effect could be detected on the percentage of abnormal sperm ($P > 0.05$), but in contrast, the effect of rooibos, as the main factor, on abnormal sperm was significant and the inclusion of 1.5 % rooibos significantly decreased the level of abnormal sperm ($P < 0.05$).

Dietary FO and rooibos levels (as the main factors) and their interaction did not significantly ($P > 0.05$) influence hatchability while the interaction between them ($P < 0.05$) affected fertility. The fertility percentage significantly increased by dietary rooibos as main factor so that the fertility level was 81.83 % and 83.67 % in 1.5 and 3 % rooibos inclusion levels respectively, this percentage was 74.5 % ($P < 0.05$) for control birds. Considering the interaction of FO × rooibos, the highest percentage of fertility was observed in birds fed on diet containing 2 % FO and 3% rooibos. Mean body weight (BW) of the birds in different treatment groups showed no significant differences. There was no interaction of FO and rooibos on BW ($P > 0.05$). Testes absolute and relative weight was significantly increased ($P < 0.05$) by inclusion dietary FO and increasing level of rooibos but their interaction was not significant ($P > 0.05$). Relative testes weight of birds fed on diet containing 3 % rooibos were 5.89 % and 2.38 % greater compared with the control and 1.5 % rooibos groups, respectively.

Discussion

In the current study antioxidative properties of rooibos, with or without FO, was included in the diets of the aged roosters to decrease the age-related subfertility. Dietary n-3 PUFAs were successfully transferred into spermatozoa and F2R0, F2R1.5, and F2R3 groups had the greatest amount of EPA and DHA ($P < 0.05$). In a previous study, a decline in the proportion of DHA in sperm with aging was suggested (Cerolini et al., 1997). Recently Safari ASL Et Al. (2018) showed that this can be compensated with supplementation of dietary FO which significantly increases DHA and total n-3 FAs ($P < 0.05$).

In this study, sperm concentration, semen volume, and abnormality of sperm were not affected by the dietary FO with or without rooibos. This may be attributable to the age of roosters in this study (52 to 64 wk) and as they kept aging through the experiment. This finding implies that reduction in sperm concentration in aged birds could not be reversed by dietary PUFAs (Akhlaghi et al., 2014b; Safari ASL Et Al., 2018). Other reproductive variables including motility and viability of sperm, and fertility were considerably improved after supplementing the diets with FO and 1.5 and 3 % rooibos. This is in agreement with the research conducted on young (Feng et al., 2015) and aged (Safari ASL Et Al., 2018) roosters in which the diets were supplemented with linseed oil and FO respectively; their results showed improvements in semen quality and reproductive performance. While our results were in contrast with that of Kelso et al. (1997) who reported no additional effects of PUFAs supplementation on sperm parameters.

Results from the current study showed that EPA and DHA from dieatry FO can transfer into the sperm plasma whilst reducing the linoleic and arachidonic content. According to Zanini et al.'s (2003) findings, dietary n-3 PUFAs increase the content of DHA and subsequently, membrane integrity in sperm. Despite of being highly effective in altering FA composition of sperm and improving membrane flexibility, PUFAs are highly susceptible to lipid peroxidation and higher MDA production (Alvarez and Storey, 1995; Baumber et al., 2000) the results of which reflexes in the sperm membrane by turning it to be also highly susceptible to oxidation that in trun leads to low sperm motility and damage to sperm integrity (Emamverdi et al., 2015) and ultimately causes fertility dysfunction (Cecil and Bakst, 1993). MDA is a posterior product in PUFA oxidation which turns it to a useful alternative in measuring the level of lipid oxidation; and as the method of measurement its reaction with thiobarbituric acid is considered as a relatively more rapid, inexpensive and sensitive technique (Valenzuela, 1991). The effect of dietary FO, rooibos and their interaction was significant in seminal plasma MDA concentration. The lowest mean MDA value was observed in birds receiving the rooibos (as main factor) 1.5 and 3 % levels compared to control group ($P < 0.05$). When the FO and rooibos interaction considered, the lowest MDA level was recorded for birds that recieved only 3 % rooibos without any dietary inclusion of FO. The MDA level was higher in birds fed on diet containing 1.5 and 3 % rooibos with FO but these levels were significantly lower in contrast to birds that received control or only FO diet ($P < 0.05$).
One of the most important findings of our study was the fertility percentage which increased when roosters fed on diet containing FO with 3 % rooibos. The FA composition and resistance to oxidation of spermatozoa may be an important predictor of fertility as the hens received the same dose of spermatozoa during AI (Cerolini et al., 1997; Akhlaghi et al., 2014b). Various studies have shown the effect of different antioxidant feed additives on increasing antioxidant capacity of seminal plasma (Golzar adabi et al. 2006, 2011; Akhlaghi et al., 2014a; Safari ASL Et Al., 2018). We used rooibos in our study because it could offer a measure of protection against induced oxidative damage by increasing the antioxidant defense mechanisms and thereby improving the sperm quality and function (Awoniyi et al., 2012). Other studies showed improvements in sperm concentration, viability and motility in male rats fed with rooibos, which might be attributed to its high level of antioxidants (Monsees and Opuwari, 2013; Opuwari and Monsees, 2014).

The results of the current study are in line with previous results that dietary inclusion of n-3 PUFA (especially in terms of EPA and DHA) can improve the roosters' fertility. So, inclusion of FA matrix of raw material and n-3 PUFA specially in aged rooster whilst feed formulation for boiler breeder roosters seems to be an essential step to be taken, roosters seems to be a very effective but at the same risky step to be taken because, it increases the sensitivity of sperm membrane to lipid oxidation. As a result, using a proper antioxidant is a good choice for preventing and/ or decreasing oxidation effect of n-3 PUFAs. Based on the results of the current study, dietary rooibos supplementation (3 %) can be considered as an influential factor in improving fertility by enhancing antioxidant status. It is not easy to give a subtle recommendation due to factors that in practical terms influence the quality of rooibos cultivated under different environmental and agricultural conditions (e.g., temperature, humidity, soil, growth stage of plant). However, rooibos can be suggested as a feasible alternative to remedy the attenuation of subfertility in aging flocks while safely eluding the biosecurity issues (e.g., spiking) and stress to the birds.

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Table 1. Means of semen parameters, fertility, hatchability, BW, absolute and relative testes weight from rooster fed diets with the different levels of FO and Rooibos[1]

Treatments	Concentration, 1×10^9	Semen volume, mL	Motility, %	Livability, %	Abnormal, %	MDA, (nmol/mL)	Fertility, %	Hatchability, %	Initial live BW, g	Ultimate live BW, g	Testes weight, g	Relative Testes Weight/ BW, g / 100 BW
Fish Oil												
0	3.69	0.49 a	0.83	0.77	0.109	2.02 b	78.67	80.89	4750.5	5167.0	26.83 b	0.518 b
2	3.80	0.48 b	0.85	0.76	0.110	2.82 a	81.33	81.91	4750.7	5173.2	29.22 a	0.566 a
SEM	0.07	0.005	0.003	0.004	0.003	0.14	1.02	1.10	3.88	11.5	0.11	0.002
P-value	0.14	<0.0001	0.36	0.49	0.11	0.03	0.08	0.52	0.97	0.70	<0.0001	<0.0001
Rooibos												
0	3.15 b	0.48 b	0.83	0.73 b	0.109 b	2.99 a	74.50b	79.25	4750.7	5173.0	27.19 c	0.526 c
1.5	4.03 a	0.48 b	0.84	0.78 a	0.108 c	1.82 b	81.83a	82.37	4750.6	5172.1	28.04 b	0.544 b
3	4.06 a	0.49 a	0.86	0.78 a	0.110 a	1.65 b	83.67a	82.58	4750.5	5162.2	28.84 a	0.557 a
SEM	0.08	0.006	0.004	0.004	0.004	0.17	1.25	1.35	4.75	14.1	0.13	0.002
P-value	<0.0001	0.002	0.74	<0.0001	0.008	<0.0001	<0.0001	0.17	0.99	0.91	<0.0001	<0.0001
FO × Rooibos												
F0 R0	3.27	0.49	0.83 c	0.75 b	0.106	2.73 a	75.67 bc	79.33	4750.6	5171.5	25.96	0.501
F0 R1.5	3.88	0.48	0.83 c	0.78 a	0.110	1.78 bc	79.67 abc	80.69	4750.7	5169.1	26.85	0.522
F0 R3	3.93	0.50	0.84 bc	0.77 a	0.111	1.56 c	80.67 abc	82.66	4750.2	5160.3	27.66	0.532
F2 R0	3.03	0.46	0.83 c	0.71 c	0.113	3.25 a	73.33 c	79.17	4750.8	5174.4	28.42	0.550
F2 R1.5	4.19	0.49	0.85 ab	0.78 a	0.107	1.86 b	84.00 ab	84.06	4750.6	5175.1	29.22	0.566
F2 R3	4.18	0.48	0.87 a	0.79 a	0.110	1.75 bc	86.67 a	82.50	4750.7	5170.1	30.02	0.582
SEM	0.12	0.009	0.006	0.006	0.005	0.24	1.76	1.92	6.72	19.9	0.18	0.003
P-value	0.90	0.20	0.004	0.01	0.20	0.029	0.0002	0.58	0.99	0.98	0.96	0.64

[1] F0 R0= control diet with no rooibos and/or fish oil; F0 R1.5= containing only 1.5% rooibos; F0 R3= containing only 3% rooibos F2 R0= containing only 2% fish oil; F2 R1.5 containing 2% fish oil and 1.5 % rooibos; F2 R3 containing 2% fish oil and 3 % rooibos; BW = Body Weight

[a-b] Different letters within the same column show significant differences among the groups ($P < 0.05$).

O[28] Organic Chelated Minerals in Broiler and Layer Breeders: Commercial Field Studies

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Novus International Yem katkıları Ltd. Şti. Karaköy/İstanbul

Introduction

Continuous genetic progress in broiler and layer breeders with a deep focus on selection, hen and progeny health status and use of high performing feed and nutritional technologies resulted in tremendous improvement in hen and progeny performance and consequently call for fine tuning especially in diet formulation for some of the micro minerals. Trace minerals, such as Zn, Cu, and Mn, function as both enzyme cofactors and constituents of metalloenzymes. These minerals protect cells from the harmful effects of free radicals by their involvement in antioxidant enzymes, such as superoxide dismutase [1]. Zinc plays an integral part in the synthesis of functional proteins, collagen as the major structural protein of internal tissues, including cartilage and bone while keratin as the structural protein of the feathers, skin, beak, and claws) [2]. Copper plays a role in the proper cross-linking of collagen and elastin [2-4], whereas Mn is essential for the maintenance of bone mineralization [5]. However, the requirements for Zn, Cu and Mn are poorly defined in most species, and our knowledge of the relationship between a mineral source and its ability to meet specific requirements lacks precision. Research has documented that mineral bioavailability varies considerably between sources (oxide vs. sulphates), for example, Zn bioavailability of ZnO has been shown to be only 44% of $ZnSO_4$ (6). As a result, inorganic trace minerals (ITM) are included in poultry diets at much higher levels of the NRC recommendations [7] (Table1). More recently, interest has been directed from ITM sources to organic trace mineral (OTM) sources with respect to bioavailability. Richards et al. [8] showed that the bioavailability of chelated Zn is between 160 and 250% that of inorganic Zn as sulphate. It was also demonstrated that commercial levels of ITM can effectively be replaced with reduced levels of Zn, Cu, and Mn of 2-hidroxy-4-methylthiobutanoic acid (HMTBa), without compromising bird performance while achieving a significant improvement in footpad health and a reduction in trace mineral concentrations in the litter [9]. Consequently, the increased bioavailability of chelated trace minerals has been attributed to reduced antagonistic reactions with other dietary constituents in the gastrointestinal tract.

Table 1. Breeders' Zn, Cu and Mn recommendations based on different sources

Requirements, ppm in feed		Zn	Cu	Mn
	Ross 308 (2016)/Cobb 500 (2013)	110	10-15	120
Added	Lohmann (2017)	60	5-10	100
	Hy-Line (2018)	100	19.30	100
Required	Rostagno (2017)	65.05	9.97	70.4
	(Organic Source)	(28.91)	(4.34)	(31.08)
	NRC (1994)	56	?	25
EU max limits for poultry (Raw materials + added)		120	25	150

Bioavailability Issue of Trace Minerals

It is very well documented that the bioavailability of ITM such as Zn, Cu and Mn is extremely affected by source of the minerals (oxide, sulphate or carbonates), type and constituents of the diet. Minerals from sulphate forms have been shown to be more bioavailable compared to oxides and carbonates forms. Many important interactions exist among various inorganic minerals that referred to as mineral antagonism under gastrointestinal tract conditions. Many minerals are therefore influenced by interaction with 2-6 other major minerals. For example, Cu antagonists are Sulphate, Zn and Mo, whereas Mn antagonists are Ca, P and Fe [10] (Figure 1). Excess amount of calcium and phosphorus presence in the diet interferes with Mn and Zn absorption by reforming in a flocculent precipitate of calcium phosphate and thereby adsorbing these minerals and carries it through the intestinal tract and impair their absorption. The main site of mineral absorption has been reported to be lower duodenum through to upper jejunum. Therefore, Mn bioavailability might decrease down to1-3% in some cases. Colloids such as of clay, insoluble salts of Al, Mg, Fe and other minerals are strongly adsorptive of cations, and this adsorption occurs both through chemical union with highly electronegative areas of the colloid surface and through attraction of the cation by physical forces.

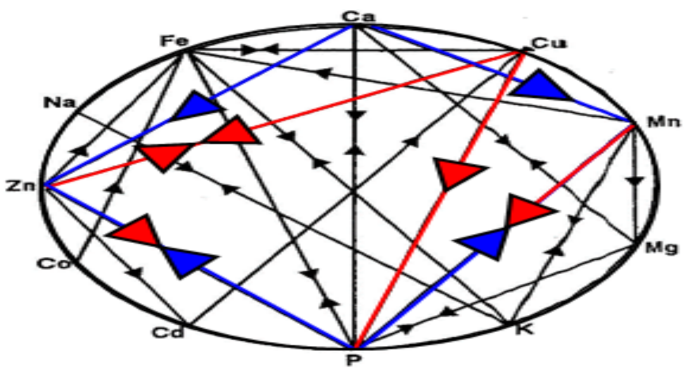

Figure 1. Mineral antagonism wheel

On the other hand, organic chelated minerals are molecules comprised of a metal and ligands attached to each other tightly. “Ligands” are the molecules like an amino acid or hydroxy acid attaches to a metal. To form a stable, chelate, multiple ligands need to connect to the metal. If only one ligand connects to the metal, it is technically a “metal complex” rather than a “chelate”. When two ligands connect to the metal, a chemical chelate is created. A bis-chelate has very specific characteristics to ensure that the metal has maximum protection. In addition to have a 2:1 ligand to metal ratio, the most important characteristic is that it must have a neutral charge. The ideal organic chelate mineral is the one that will release the mineral in ionic form at the intestinal wall, or that can be absorbed as the intact chelate. The most stable chelation rings using Zn, Cu and Mn are formed by HMTBa [11, 12]. Such a chelate may markedly enhance the absorption of a mineral by preventing its conversion to an insoluble chemical compound in the intestine, or by preventing its strong adsorption on an insoluble colloid. Some chelates holds the metal so firmly that the metal can become almost unavailable to plants or animals (phytic acid). Many chelates are highly absorptive and protect the mineral from forming an insoluble complex [10].

Commercial Field Trials

To test the effect of chelated minerals of HMTBa-MC (Mintrex® Zn, Cu and Mn, Novus International) two trials were conducted one with broiler breeders and the other one with layer breeders:

Broiler Breeder Trial

In Belgabroed Breeding farm near to Eindhoven approximately 43 000 Ross 308 breeders confined in 6 houses were given 50-10-65 ppm Zn, Cu, and Mn as Mintrex® program (in Reduce and Replace strategy) versus 100-15-100 ppm Zn, Cu and M as inorganic sources (sulphates) in comparison with historical data (2015) and company average as benchmark. Trial lasted for 40 wk (from 20 wks of age until 60 wk) in 2016.

Results of the Trial 1 (Broiler Breeders Ross 308))

Based on an additional reference line using the following formula: (Age of the hens in weeks + laying rate (%))* 100, the Breeder Company generated a graph for laying rate and above 120 (the reference point) was considered to be a very good performing herd. When the plateau was reached at week 29, HMTBa-MC fed birds continuously exceeded this level, meaning that in the group fed with organic chelated minerals (HMTBa-MC) hatching egg production was improved and exceeded continuously the reference line (Figure 2).

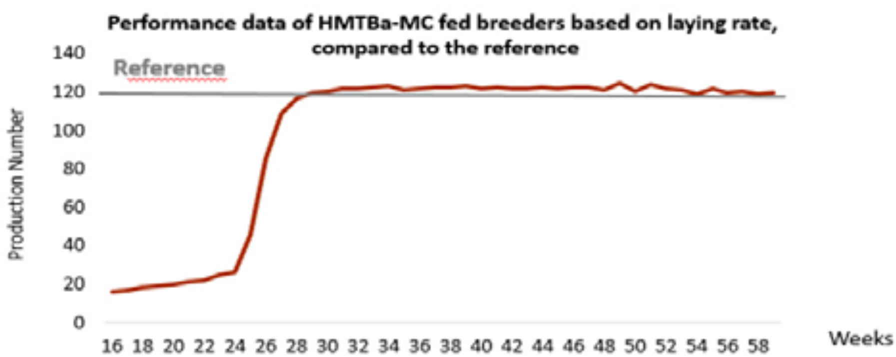

Figure 2. Effect of HMTBa-MC on breeder’s hen laying rate in comparison to reference line

Likewise, hatchability was significantly improved by HMTBa-MC vs ITM and company benchmark and increased from 83.74% to 85.17% in the organic chelated minerals fed group (HMTBa-MC) (Figure 3).

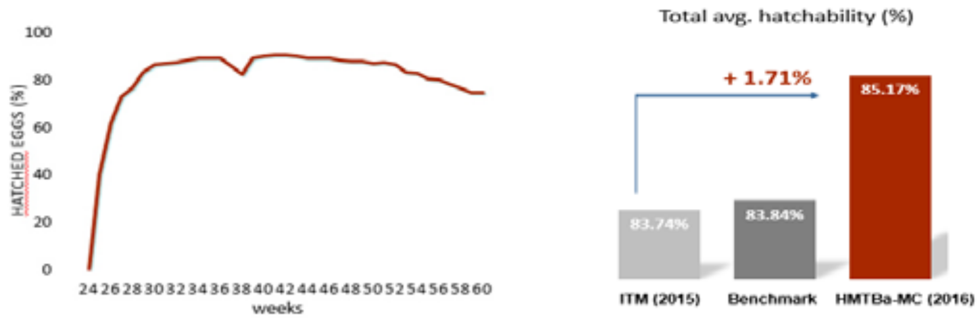

Figure 3. Average hatchability of broiler breeders fed HMTBa-MC vs ITM and company benchmark

In the hatched eggs graph at week 37/38 the hatchability numbers were affected due to an avian pneumovirus (APV) infection, typically lowering laying performance and egg shell quality. However, this depression in laying rate and hatchability was compensated during the later stages of this trial. Average number of cracked eggs were also decreased by 2% from 5.63% to 3.56% in the group fed with HMTBa-MC vs. ITM fed group (Figure 4).

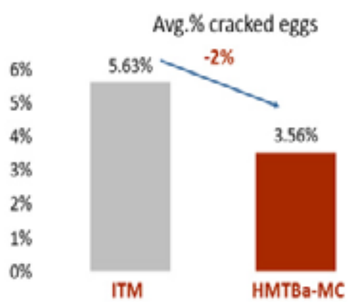

Figure 4. Effects of HMTBa-CM on average cracked eggs ratio

The increase in egg shell quality can be attributed to higher bioavailability of organic chelated mineral source compared to ITM as a consequence of more efficient supply of Zn, Cu and Mn to the hen`s metabolism more minerals present in body for key enzymes involved in calcification process during egg shell formation [2,12].

When comparing the number of cracked eggs by HMTBa-MC fed houses vs. historical data, the amount was significantly lower in 5 out of 6 houses compared to the so-called 25% egg quality benchmark (EQB) which represents the 25% lowest percentage of cracked eggs achieved by the houses over the previous years.

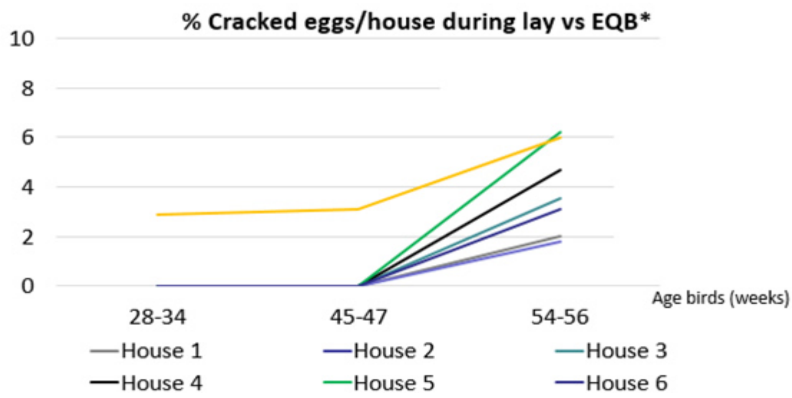

Figure 5. Percent of cracked eggs /Breeder House during lay (EQB)

The effects of supplementation of organic chelated minerals (HMTBa-MC) to broiler breeder diets decreased mortality and numerical decrease from 0.96% to 0.86% were observed with respect HMTBa-MC group vs ITM. Consequently, the profitability analysis of the broiler breeder trial indicated that breeders fed with HMTBa-MC supported bird performance and resulted in improvement of number of DOC/HH by 0.8%, decreased feed intake/DOC by 3.1%, decreased feeding cost/DOC by 4.9%, increased income/HH by 0.8%, increased feeding profit by 2.3% and consequently and resulted in return/HH 0.853€ and ROI as 12.641 (Table 2).

Table 2. Effects of feeding organic chelated minerals and ITM on broiler breeders flock profitability

Table 2. Effects of feeding organic chelated minerals and ITM on broiler breeders flock profitability

Parameter	ITM	HMTBa-MC	Improvement
Number of Day Old Chicks (DOC)/hen	141.09	142.22	0.80%
Feed Intake/DOC	0.33	0.32	-3.10%
Feeding cost/DOC (€)	0.08	0.076	-4.90%
Price DOC (€)	0.34	0.34	-
Income per HH	47.97	48.35	0.80%
Feeding Profit (€)	36.64	37.49	2.30%
Feed investment/HH	0	0.067	
Return/HH €		0.853	
Return Of Investment		12.641	

Conclusion

Supplementation of HMTBa-MC in broiler and layer breeder diets in Reduce&Replace strategy at of 55-10-65 ppm levels respectively for Zn, Cu and Mn to replace higher inclusion levels of ITM or ITM+OTM significantly improved breeder performance and hatching results in terms of laying rate, hatchability, decrease in cracked eggs, increase in viability of embryos during longer storage of hatching eggs.

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IS[15] Why Infectious Bronchitis And Newcastle Disease Vaccinations Can Not Be Protective? Practical Importance of Genotype Selection

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Infectious Bronchitis (IB) and Newcastle Disease (ND) are two major respiratory infections in poultry sector in Turkey and in the World. Agents of these infections are IB virus (IBV) and ND virus (NDV), respectively. NDVs affect primarily respiratory and digestive system together with all tissues and organs, although IBVs show their effects on respiratory, reproductive and urinary systems. Based on the effects of these viruses, clinical symptoms and pathognomonic lesions develop in the affected chickens, with declines in production parameters, and increases mortality rates. Diagnosis of the two infections is performed by isolation and identification of the viruses from clinical samples. Conventional procedures, such as virus neutralisation, or molecular techniques such as polymerase chain reaction (PCR) are used to identify the viruses. PCR modifications are also used to detect the viruses directly from clinical samples without any need for virus isolation. Beside detection of the viruses, ND diagnosis also needs additional pathogenicity tests to determine whether NDV is in the velogenic or mesogenic pathotypes, which are responsible for clinical disease. Commercial and breeder flocks are largely protected from these diseases by using different vaccines and following vaccination strategies. In this context, it should not be forgotten that primarily induced-local or mucosal immunity, and then induced-systemic immunity by live vaccines play crucial roles both in the protection against these infections, and in reducing shedding from possibly infected chickens with field virus. Obstructive factors responsible for developing the vaccine-induced protective immunity can be considered in two classes: 1. Vaccine virus-related (virus genotypes), and 2. Other factors (immunosuppression or malpractices in vaccinations). Besides vaccination malpractice and immunosuppressive agents, individual genetic and antigenic changes in the NDV and IBV cause major problems with protection against their infections all over the world. In this presentation, first I will introduce the structural components of the viruses, which represent changes, and how these changes could constrain the efficacy of vaccinations in practice. Then, I will cover the detection of these viruses, and emphasize on the methods for detecting and analysing to determine the changes in variant IBV strains and NDV genotypes circulating in different regions or other countries. Finally, I will discuss what we could do about vaccinations against IBV and ND infections in our country.

O29 Investigation of Newcastle Diseases Virus in Wild Birds Species Around Küçükçekmece Lake

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Abstract

In the present study, passeriform and nonpasseriform birds and ducks were investigated for the presence and genotypes of NDVs.   For this purpose, field studies were performed with birds migrating on the South East European flyway, in the Marmara region of Turkey which borders the European Union. Traps were placed around the Kucukcekmece lake Avcilar, Istanbul, in the spring season of 2016 and 2018 to catch passeriform and nonpasseriform birds. The trapped birds were categorized according to species and sex, ringed and oropharyngeal and cloacal swabs were taken. In total, from 42 different bird species, 297 oropharyngeal and 297 cloacal swabs were collected. In addition, in 2018, swabs from 115 mallards (*Anas platyrhynchos*) were sampled by hunters in the Edirne area in Turkey. Also, swab samples from 31 species of birds (n=207) treated at the Wild life clinic at the Veterinary Faculty of Istanbul were analyzed.

Real-time RT-PCR analyses was ferform for the presence of NDV genetic signatures. Positive samples were further subjected to sequencing. Pyhlogenetic analyses were performed to determine genotypes NDV in the targeted bird population. NDV-specific RNA was found by real time RT-PCR in 5 wild birds. When sequencing and phylogenetic analysis performed, all ND viruses were belong to the NDV lineage VII. Results of this study indicate that migratory birds present a threat for Turkey to spread NDVs.

Key words: Newcastle disease virus, wild birds, RT-PCR, genotypes, phylogenetic, Turkey

O[30] Bdellovibrio and Predatory Bacteria: a New Opportunity in *Salmonella* Control?

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Abstract

The presence of predatory bacteria was selectively analyzed in metagenomic data obtained from studies investigating the association of gut microbiome with *Salmonella* infection of broilers. All data collected from *Salmonella*-infected and -free chickens were included in the analysis. For this purpose, bacterial 16S rDNA sequences obtained from two next generation sequencing platforms, namely IonTorrent PGM and Oxoid Nanopore were analyzed by metagenomic tools. Predatory bacterium *Bdellovibrio* was detected in the gut samples of 24 chickens. *Bdellovibrio* classification was confirmed by high homology scores to reference sequence. While *Bdellovibrio* was found in 23 of 48 (47.9%) *Salmonella* negative samples, it was found in only one of 17 (5.8%) *Salmonella* positive samples. The relative abundance of *Bdellovibrio* was correlated with that of *Proteobacteria* phylum. A negative correlation was observed, however, between the relative abundances of *Enterobacteriaceae* family and *Bdellovibrio*. It was suggested that *Bdellovibrio* may affect the *Salmonella* infection in chickens in natural settings and may be used to control the infection.

O31 Promoting Factors of Turkey Meat Products Oxidation

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Introduction

Poultry is consumed on a worldwide basis and, in recent years, has risen drastically due to its lower prices compared to red meats. To ensure the continuous growth and competitiveness of the poultry industry it is essential that meat quality and safety are maintained during production and processing (1). Turkey meat is known for being one of the healthiest meats, containing less calories and fat compared to chicken, beef and pork (2). Turkey meat is very versatile when it comes to cooking: it can be prepared in various ways such as roasted, fried, grilled, stuffed, among others. One of these ways is like turkey skewers (TS), adding vegetables between the meat, like tomato, onion, green pepper, pineapple or bacon and chorizo. TS are usually grilled; this way of cooking is seen all over the world, with slight differences in the ingredients used or in the way which are cooked. More recently, in order to respond to consumers demands and to preserve their nutritional and physic-chemical characteristics delaying deterioration, this poultry product have been commercialized in retail large surfaces packaged in modified atmosphere (1). Fresh meat preservation is a problem that concerns all meat industries because oxidation is a natural process of its degradation and, at the present, it is only possible to slow it. The ultimate aim of the industry is to delay the deterioration of meat in order to preserve its characteristics as long as possible with all available means, in the most efficient and economical way, avoiding loss of commercial value and food waste. Some ways of preserving the physicochemical characteristics of meat are the application of modified atmosphere packaging, low temperatures and suitable packaging materials. The main objective of this work was to study the promoting factors of turkey meat products oxidation. With this purpose, three assays were carried out to determine the influence of the packaging material, atmosphere/meat *ratio* and storage light on turkey skewers oxidation.

Materials and Methods

Assays Effect of Packaging Material (F), Atmosphere/Meat *Ratio* (C) and Light (L) on turkey meat products (Skewers)
For the assay related to packaging material (F) it was used two different films (non-printed, F1, and printed, F2) to study its effect on the product along shelf-life period (7 days at 3 ± 2ºC). F1 was a laminate film based on polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP) and F2 a laminate based on PE, ethylene vinyl alcohol (EVOH) copolymer and polyamide (PA). Greater difference between films was oxygen transmission rate (OTR): F1 – 53,9 $cm^3/m^2/24h$ and F2 – 5 $cm^3/m^2/24h$. In the second assay (C), to assess the influence of the atmosphere/meat *ratio*, two different packages were analyzed, one with four skewers (C1) and an atmosphere/meat *ratio* of 1,16 and another package (C2) with three skewers with an atmosphere/meat *ratio* of 2,11. The objective of this assay was to evaluate the influence of

the available protective atmosphere on product colour (promotion or delay of discoloration). The package used on this assay had the same characteristics of the package used on F1 assay. The third assay was performed to evaluate the effect of light on the product (L) along shelf life period (7 days at 3 ± 2ºC). Turkey skewers were exposed to different types of light, in the refrigeration room with standard light conditions, simulating retail display (L1) and stored at similar temperature conditions, but in the absence of light (L2). Characteristics of the light used in the refrigeration room (L1) were: LED Tube T8 25W - 1500mm Frosted 6000K; Wattage 25W; Voltage AC100-240V; type of LED, SMD (surface mounted device); light beam angle 120º (degrees); luminous flux of 2450lm; light colour of 6000K; cover of frosted plastic; and the dimensions of each light structure – length: 1500mm; width: 26mm. The package used on this assay had the same characteristics of the package used on F1 assay.

Turkey Meat Products Processing Technology

Turkey meat was provided from national suppliers (Portuguese slaugheter houses) as refrigerated carcasses to be deboned at the industry. From the carcass was removed the thigh and the meat, bacon (Paranho Carnes, Portugal) and green peppers (ELS, Portugal) were spiked, interchangeably, on wooden sticks by an operator and sliced on a cutting machine (Bucelmaq, Portugal). TS were packaged under MAP in EPS cuvettes (Coopbox, Italia) with absorbent pads and thermo-sealable laminate (Ronzulli, SpA, Italia; Bemis Packaging Solutions, USA). Skewers were packaged in a horizontal wrapping machine (Mondini Proface, p. A, Italy) and the protective atmosphere applied was 70% O_2:30% CO_2 (Air Liquide, France). After packaging, samples were stored at 3 ± 2 ºC, until expedition order.

Analysis

Tightness Test and Temperature Control

The tightness test was carried out on all samples, after packaging, to check for any defects like poor sealing and pinhole punctures that may occur at the time of packaging. The test was performed by dipping the packages into a container of sufficient size full of water so that it fitted completely submerged, and deformations and leaks were detected through release of bubbles from the package. The temperature was measured daily along shelf-life period (7 days), on two samples, by penetration with a thermometer (Etiltd, UK) after measurement of atmospheric composition.

Microbial Analysis

Samples were analyzed at day 0, 3, 5 and 7 (day 0 was the packaging day). On each sampling day, three samples were analyzed (n=3). The preparation of the samples was carried out in accordance with ISO 6887-2 (3). The analysis proceeded by aseptically remove 10g of sample to a sterile Stomacher Bag (Seward, UK) containing 90ml of tryptone salt (0.1% w/v) (Oxoid, England) and blend in the stomacher during 30 seconds. The 10g of sample were composed of thigh meat, pepper and bacon portions, taken from different sections of the skewers, obtaining a homogeneous and representative mixture. Preparation of dilutions were performed according to NP 3005:1985 (4). Mesophilic counts were performed by spread plated on plate count agar (PCA) (Biokar Diagnostics, France) and incubated at 30ºC for 3 days (5). Lactic acid bacteria counts were determined by sample incorporation on Man, Rogosa and Sharpe agar (MRS) (Biokar Diagnostics, France), incubated at 30ºC for 3 days (6). Yeasts and moulds were enumerated on Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) (Oxoid, UK), and incubated for 5 days at 25ºC (7). Coliforms counts were determined by sample incorporation on Violet Red Bile Lactose Agar (VRBL Agar) (Oxoid, UK), incubated at 37ºC for 24h (8). *E. coli* counts were determined by sample incorporation on Tryptone Bile X-Glucuronide Agar (TBX Agar) (Merck, USA), incubated at 44ºC for 24h (9). All microbiological analysis were carried out in triplicate (n=3) and the results were converted to a logarithm of the number of colony-forming units (CFU/g). Horizontal method for the detection of *Salmonella* spp (10) and Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp (11) were carried out.

Measurement of atmospheric composition

Atmospheric composition (% O_2 and % CO_2) (n=1) was determined along shelf-life period (7 days), every day, with a gas meter (Checkpoint O_2 / CO_2 meter, Dansensor, Denmark), at room temperature (approx. 25ºC). Results were expressed in gas percentage.

Measurement of pH

It was measured daily (n=3) along shelf-life period (7 days) with a meat pH meter, glass electrode (HANNA Instruments, USA), at room temperature (approx. 25ºC).

Measurement of a_W

Water activity was measured along shelf-life period (7 days), in the three components of the TS samples (n=3) (meat, bacon and pepper pieces) with a meat a_W meter (Hygropalm - HP23-AW-A, Rotronics, France) at room temperature (approx. 25ºC).

Measurement of Colour

These parameters were measured in the first and last sampling days (day 0 and day 7). The colour was measured with a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan) (n=3), with the CIE L* a* b* system, where L* is a measure of the lightness (0 being the darkest and 100 being the lightest), a* is a measure of redness, ranging between green (-values) and red (+values), and b* is a measure of yellowness, varying between blue (-values) and yellow (+values). All colour measurements were carried out ten times, at different places of the skewers.

Measurement of TBARS

Thiobarbituric Acid Reactive Substances (TBARS) were measured following a Demiral and Turkan (12) adapted protocol (n=3). All TBARS measurements were carried out in triplicate on day 0 and day 7. The analysis proceeded by aseptically taking a portion of meat from the package and grinding it in a meat grinder (Northern Tool + Equipment, USA) removing 0,5 g of sample to a mortar containing 2,5 mL de 0,1 % (p/v) TCA (0.1% solution of trichloroacetic acid in deionized water). With a pestle the mixture was homogenized as best as possible. 1500 µL were then centrifuged at 12 000 x g (Sigma-Aldrich, USA) during 15 minutes. After that 1 mL of supernatant was mixed with 4 mL of 20% TCA + 0.5% TBA solution (20% solution trichloroacetic acid and 0,5% thiobarbituric acid in deionized water) proceeding to heat at 100ºC for 30 minutes followed by rapidly cooling in an ice bath to stop the reaction. After a few minutes the solution was centrifuged at 10,000 x g for 15 minutes finishing with absorbance reading in a spectrophotometer (Analytik Jena, Germany) at 532 nm and at 600 nm in glass cuvettes. The results were expressed in millimolar (mM) of malondialdehyde (MDA).

Statistical Analysis

Statistical analysis was performed using the software R for parameters O_2, CO_2, pH and a_W. Differences between mean values were evaluated by a *t*-test to verify the difference of mean values between samples (*p*-value > 0.05) and the assumptions of normality were verified using the Shapiro-Wilk test (*p*-value > 0.05). For the results of colour parameters, TBARS and microbiology it was not possible to proceed with statistical analysis and results were only organized as $\bar{x}\pm SD\bar{x}\pm SD$ in graphs, for microorganisms and, in tables, for colour parameters and TBARS.

Results and Discussion

The parameters that showed greatest discrepancy among samples were the packaging gases, oxygen (O_2) and carbon dioxide (CO_2) (Figure 1), the color parameters a * and b * and TBARS (Table 1).

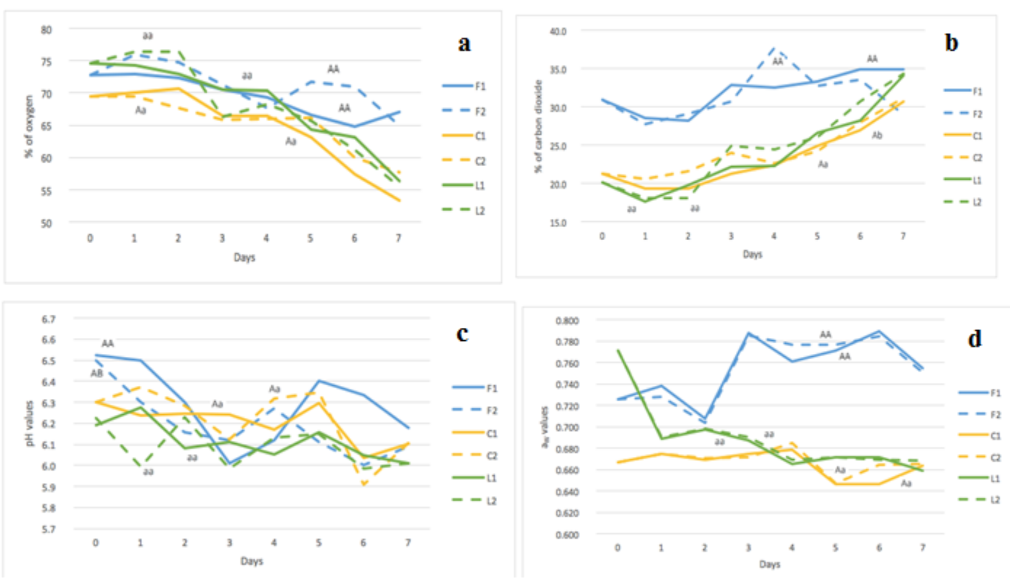

Figure 1. Evolution of O_2 (a), CO_2 (b), pH (c) and a_W (d) along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C1 atmosphere/meat *ratio* of 1,16 and C2 of 2,11; L1 storage with light and L2 storage without light. For each assay same pair of letters means that results do not differ significantly (*p*-value>0.05); Assay F (uppercase letters); Assay C (upper and lowercase letters); Assay L (lowercase letters).

Regarding the parameters a_w, L* parameter and microbiological counts (Figure 1-2, Table 1), the differences were less relevant, and for a_w were obtained very similar values for all samples from the different assays, and there were even samples with equal values , as in the case of measurements on day 5, where the values for assay C (atmosphere/meat ratio), were the same and very similar to those observed on assay L (light conditions), (a_wF1 = 0.771 , a_wF2 = 0.777, a_wC1 = 0.647, a_wC2 = 0.647, a_wL1 = 0.672, a_wL2 = 0.671) (Figure 1). Assay F, which compares two types of packaging material, were observed differences between samples, mainly in O_2, CO_2 and TBARS (Figure 1 and Table 1). Printed film (F2) recorded an irregular variation in CO_2 content along shelf life period period (Figure 1) and samples of F1 and F2 showed pH values significantly different (p-value <0.05) (Figure 1). Regarding color parameters and TBARS (Table 1), differences were evident between F1 and F2 with a more pronounced degradation of F2 showing formation of higher lipid oxidation byproducts (F2TBARS = 1,533; F1TBARS = 1.137). For the remaining assays it was not observed such differences between samples, meaning that the variables atmosphere/meat *ratio* and light conditions along shelf life period did not significantly influence the product. Nevertheless, it was possible to conclude in assay of atmosphere/meat *ratio*, that the package with a 2.11 (C2) *ratio* presented a higher value for parameter a* in the end of the shelf life period (7 days) (Table 1) and, for the same package, higher oxidative stability, since presented a lower value for TBARS, in opposition of packaging with a ratio of 1.16 (C1TBARS = 1.339; C2TBARS = 0.468) (Table 1).

In what concerns the study of light conditions, a marked increase in CO_2 values from day 2 to day 3 (Figure 2) was observed in samples stored without light (L2). For parameter b*, there was an irregular variation for L1 (with light), decreasing at day 7 and a slight increase for L2 (L day 0 b* = 6.13; L1 day 7 b* = 5.35; L2 day 7 b* = 6.19) (Table 1). Also, for L2, a more pronounced increase of TBARS (L1TBARS = 0.657; L2TBARS = 1.158) was observed (Table 1) leading to the belief that the absence of light promotes the oxidation of turkey meat and a slight tendency to color change (more yellowish). All samples of the 3 assays presented high values for microbiological analysis (Figure 3), reaching values higher than 7 log cfu/g on days 5 and 7 for total aerobic mesophilic microorganisms (Mots). Detection of *Eschirichia coli*, *Salmonella* spp and *Listeria monocytogenes* were negative in all samples.

Conclusion

Assay related with the packaging material showed the most relevant results, reflecting its influence on meat colour. F2 (printed film with lower OTR) revealed abnormal packaging atmosphere composition measurements in comparison to F1 (transparent film with higher OTR). The packages differed significantly from each other in relation to pH (*p*-value <0.05) and displayed relevant differences on colour and TBARS. It was observed a more rapid degradation of F2. The results obtained with the others assays (light conditions and atmosphere/meat *ratios*) did not allowed such precise conclusions to be drawn and therefore the packaging material was considered to be the factor with greatest impact on this type of turkey meat product oxidation.

Table 1. Colour (n=10, $\bar{x}$±SD$\bar{x}$±SD) and TBARS (nmol MDA/g) (n=3, $\bar{x}$±SD$\bar{x}$±SD) measured values along product shelf-life (7 days at 3 ± 2ºC). F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; C referred to atmosphere/meat *ratio*s: C1 with lower *ratio* and C2 with higher *ratio*; L1 storage with light and L2 storage without light.

	Colour							
Day	**L***		**a***		**b***		**TBARS**	
Assay F								
0	46,10 ± 4,13		15,59 ± 2,97		9,47 ± 2,11		0,431 ± 0,236	
	F1	F2	F1	F2	F1	F2	F 1	F2
7	48,61 ± 3,2	50,08 ±3,4	14,82 ± 4,97	12,74 ± 5,54	10,64 ± 1,97	10,32 ± 1,9	1,137 ± 0,30	1,533 ± 0,03
Assay C								
0	45,46 ± 3,76		15,18 ± 4,03		8,99 ± 2,55		0,168 ± 0,032	
	C1	C2	C1	C2	C1	C2	C1	C2
7	48,55 ± 4,6	49,16±5 7	13,00±4,1 1	16,33 ± 2,5	10,12± 2,8	10,31±1,8	1,339±0,6 5	0,468± ,03
Assay L								
0	44,28 ± 2,74		14,00 ± 2,75		6,13 ± 1,91		0,061 ± 0,018	
	L1	L2	L1	L2	L1	L2	L1	L2
7	47,74 ± 2,5	47,89±3 7	11,38 ± 4,4	13,37 ± 3,6	5,35 ± 1,08	6,19± 2,4	0,657±0,0 2	1,158±0,7 5

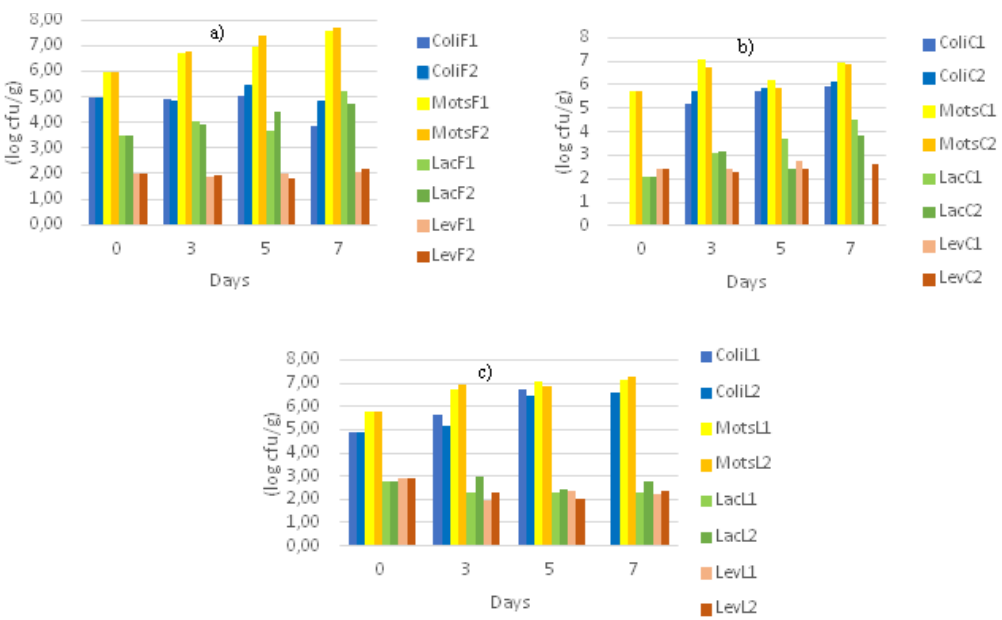

Figure 2. Microbiological analysis related to packaging material (a), F1 – transparent film with higher oxygen transmission rate (OTR) and F2 – printed film with lower OTR; atmosphere/meat *ratio* (b), C1 with ratio of 1,16 and C2 with 2,11; storage light conditions (c) L1- storaged with light and L2 - storaged without light. Coliform bacteria (coli), total mesophilic aerobic microorganisms (mots), lactic acid bacteria (lac) and yeasts (lev) counts (log cfu/g) along product shelf-life (7 days)

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O[32] Effects of Using Different Rates of Kefir in the Marination of Pre-cooked Ready to Eat Turkey Cubes

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Abstract
In this study, it is aimed to improve the flavor, texture, color properties, cooking characteristics and microbiological quality of ready-to-eat marinated and pre-cooked turkey thigh meat and to obtain a healthy product by using kefir in marination mixtures. In this context, the samples were marinated with the mixtures containing three different concentrations of kefir (15%, 30%, 45%), the samples treated with the marinated mixture without kefir were evaluated as a control. The analyzes were carried out in the turkey meat, in the marinated samples and the samples cooked after marination. In the samples, other than chemical composition (moisture, fat, ash, protein), pH, color, texture profile, marination absorption, cooking efficiency and sensory evaluation analyzes, TBARS, total aerobic plate and lactic acid bacteria analyzes were also performed to determine the effect of marination on oxidative and microbiological properties of samples. Using increased concentration of kefir in the marination mixture caused an increase in the protein values of cooked turkey meat. Kefir marination affected the color properties of the samples, caused an increase in L^* values and decrease in a^* and b^* values. The highest value of the marination absorption was determined in the sample treated with the mixture including the highest concentration of kefir (M45). The elasticity, chewiness and flexibility texture properties of the samples were positively affected by the use of kefir. The counts of total aerobic plate and lactic acid bacteria in marinated meat samples increased in parallel to the kefir concentration in the marinate, but the results of microbiological counts in these samples decreased under detection limit after applying cooking process. The results of the sensory evaluation showed that the samples treated with the mixture containing 30% kefir were the most favored samples, but there were no statistical differences between the samples. In this study, it was concluded that the use of kefir in pre-cooked ready-to-eat turkey meat will contribute to offer an alternative product to the poultry sector by improving some of the properties of the product.

Keywords: turkey meat, kefir, marination, quality

O33 Which is the Best Method for Thawing of Chicken Breast and Thigh Meat?

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Abstract

Chicken breast meat and chicken thigh meat, which have high consumption and selling rate, are primary products of poultry industry. Freezing is usually applied to preserve the quality of these products. Thawing is also effective factor for the protection of thawed meat quality and safety; therefore, it is crucial to use suitable thawing method. With that said, the chicken breast and thigh meats were freezed at -18°C and stored for 48 hours at this temperature. Then the effect of ambient air thawing (at 22±2°C for 8 h), refrigerator air thawing (at 4°C for 16 h) and microwave thawing (breast meat at 180 Watt for 7.5 min, thigh meat at 180 Watt for 8 min, tempering at 4°C for 2 h after thawing) on quality parameters of frozen chicken breast and thigh meats were evaluated. All thawing procedures resulted in decrease for protein solubility and water holding capacity. The decrease in water holding capacity is the highest in samples thawed at ambient air due to the high thawing temperature. L^* and a^* values were found to be higher in thawed breast and thigh meats comparing with unfrozen meat. Additionally, thawing triggered the lipid oxidation in all groups. Application of microwave thawing or refrigerator air thawing for frozen chicken breast and thigh meat can be suggested in order to obtain good quality meat products.

Key words: Freezing, microwave thawing, protein solubility, lipid oxidation, water holding capacity

O[34] Effect of Different Cooking Methods on Textural, Sensorial and Color Properties of Commercial Chicken Nuggets

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Abstract

In this study, the color and sensorial properties of the commercial chicken nugget (PN) samples from different brands before cooking were determined and compared and as well as their sensorial, textural and color properties after cooking by different methods (oven and fryer). The cooking loss values were in the range of 21.30-27.04% for oven cooked samples and 6.93-15.18% for the fryer cooked samples. While the hardness values of the samples before cooking varied from 5.62 to 18.75 N, this range increased to 19.46-46.43 and 19,51-39,81 N for oven and fryer-cooked PN samples, respectively. The surface color values of PN samples were in the ranges of 49.90-68.73, 4.18-13.16 and 8.64-43.71 for L*, a* and b* values, respectively. On the other hand, the L*, a* and b* values at the cross-sectional surface of the samples varied in the ranges of 62.98-76.81, 0.27-3.09 and 13.70-19.12, respectively. According to the sensory scores, all samples were appreciated by panelists; however, in general, the color, hardness and chewiness of the oven-cooked samples were higher than their counterparts.

Keywords: Cooking loss, frying, hardness, color, chewiness

O35 Effect of Addition Gilaburu (Viburnum Opulus L.) Extract At 1% and 2% Levels on Oxidative Stability of Ground Chicken Meat

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Abstract

The effect of addition gilaburu (*Viburnum opulus* L.) extract at 1% and 2% levels on oxidative stability of ground chicken meat was investigated during refrigerated storage at 4°C. Gilaburu extract incorporation resulted in lower pH values ($p<0.05$). TBARS value was significantly lower in 2% gilaburu added group than the control ($p<0.05$) indicating that gilaburu addition at 2% level could have antioxidant effect on chicken ground meats. CIE lightness (L*) values decreased with the addition of gilaburu extract. In the consumer acceptability panel, sensory attributes such as appearance, color, odor, flavor, texture and overall acceptability were evaluated and no significant difference was found between the control and gilaburu incorporated groups in terms of all sensory characteristics tested ($p>0.05$). Data obtained from the present study suggest that gilaburu extract could be used as a natural alternative to the artificial antioxidants in chicken meat products without negatively altering sensory properties.

Key Words: Chicken meat, Gilaburu (*Viburnum opulus* L.), Antioxidant activity

IS[16] Role of Amino Acids on Intestinal Function during Enteric Challenge in Broiler Chickens

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The gastrointestinal tract of a broiler chicken is always susceptible to environmental pathogens which make chickens more susceptible to dysbacteriosis, inflammation and intestinal diseases such as coccidiosis and necrotic enteritis. The intestinal function is highly affected by dietary factors, including amino acids. Amino acids play an important role in regulating intestinal function and integrity. An optimal gut integrity depends on the coordinated regulation of the mucus layer, the para-cellular tight junctions, epithelial cells, and host innate and adaptive immune response. Any disruption on enteric integrity could lead to disease.

Amino acids including essential amino acids and nonessential amino acids can improve intestinal function and integrity. Amino acids are constitutive nutrients for mucosal integrity and modulators of expression of cell growth signaling, anti-inflammatory cytokines and tight junction proteins. The antioxidant properties of certain AA prevent the apoptosis and oxidative stress of the enterocyte. Therefore, the supplementation of A.A under condition of inflammation and infection can improve gut health, growth and can ameliorate the impact of enteric diseases. In this review, we summarize the role of amino acids on intestinal function, including, intestinal differentiation, intestinal structure and immune responses, and how amino acids influence the host response under different enteric challenges in broiler chickens.

Intestinal Proliferation and Differentiation

Different amino acids play an important role in epithelial cell turnover and intestinal maintenance. Branched-chain amino acids including Leucine (Leu) and Arginine (Arg) promote increase in cell size and protein synthesis through the upregulation of a signal pathway mTOR (29). Likewise, glutamine (Gln) stimulates intestinal growth by serving as a fuel source for enterocytes (15) making the intestine the main site of glutamine uptake (44). Although glutamine is a non-essential amino acid and can be synthesized by many cells in the body, it is an important amino acid for maintaining and efficient immune response. Such functions include serving main energy source in small intestine and for immune cells including lymphocytes, macrophages and heterophils (5). Therefore, under inflammatory conditions and injury glutamine is considered a conditionally essential amino acid (25).

It has been shown that Gln promotes the intestinal cell proliferation via activation of AMP-activated kinase (AMPK) and the intestinal cell survival by blocking the enterocyte apoptosis signal (29). In chickens, the supplementation of 1% and 1.5 % of glutamine significantly increased the weight and villus height of duodenum and jejunum at 21 and 42 days (22), (3). This positive effect was also observed at early age, on 7-day-old broilers (23) and in mature birds. A recent study showed the proliferative activity of L-glutamic acid (Glu) in laying hens.

When 69-wk-old hens were feed with 2.88 to 3.08% of Glu, an increase in the size of jejunal villi was observed and the proliferative cell nuclear antigen was positive on supplemented treatments (26).

In addition, the hydrolyzation of Gln promotes glutathione production, which is an important molecule in oxidative stress (43). The dietary supplementation of glutamine (1%) in early weaned piglets upregulated the expression of genes that prevent the oxidative stress and enhance cell growth (41).

Moreover, Arginine (Arg) plays an important role by stimulating intestinal development (40). In the enterocyte, about 40% of dietary arginine is converted to citrulline. Similarly, arginine can be metabolized into ornithine essential for the formation of proline and polyamides, molecules directly associated with cell viability, cell growth and cellular differentiation and proliferation of the intestine (10).

Amino Acids and Epithelial Barrier Function

Maintaining an optimal intestinal barrier function is critical for preventing pathogens and toxic compounds to cause enteric infections (2). The intestinal epithelial barrier consisted of a continuous monolayer of intestinal epithelial cells bounded by intercellular junction complexes (14). The link between cells serves as a first line of defense in the intestine preventing microbial colonization from the luminal side. Any alteration of tight junctions results in a disruption in intestinal homeostasis predisposing to enteric diseases (2).

In the recent years, multiple studies have shown the important role of amino acids in the regulation of the expression of tight junction proteins. *In vitro* studies have shown that glutamine starvation of human caco-2 cells increased intestinal permeability and reduced the expression of tight junction proteins (8). In intestinal porcine epithelial cell line, the supplementation of glutamine (gln) increased the mRNA expression of Zona occludens 1 (ZO1) gene in 47% (46), a crucial membrane structural protein involving in tight junction (39). Likewise, other amino acids such as threonine and tryptophan enhance the expression of tight junction proteins. In a chicken lipopolysaccharide inflammatory induced model, the supplementation of threonine (3 mg/kg) upregulated the expression of ZO1. Other proteins including ZO1, occludins and claudins that play a critical role in tight junction regulation are upregulated by 0.2% tryptophan supplementation in weaned pigs (17).

Amino Acids and Immune Secretory System

The mucosal surface is regularly covered by intestinal mucus, complex lipopolysaccharides secreted by goblet cells (11). This mucus layer and secretory IgA (sIg) represents the first line of defense against physical and pathological injuries (20). Mucins are the main component of mucus and are large glycoproteins with protein backbone structure composed mainly by cysteine, proline, serine, and threonine (13). In fact, threonine represents 28% of the amino acid composition in mucin (30). The high incorporation of threonine on intestinal mucins (70%) is reflected by the high utilization of threonine by the intestine and liver (62%) (35). The secretion or disruption of intestinal mucins have a direct impact on the maintenance requirement for threonine. Therefore, an increased mucus production will consequently increase the threonine requirement (34). During inflammation, threonine availability is reduced due to its high demand for synthesis of mucins (33).

Furthermore, threonine is a key component of the enteric secretory system representing 7 to 11% of total amino acids of immunoglobulin A (30). The secretory immunoglobulin A (sIgA) plays an important role in blocking pathogen adhesion, protection the animal from intestinal and luminal antigens. Due to the high threonine content of immunoglobulins and mucin, threonine is a critical amino acid in modulating the immune function and in protecting the gut wall from injury and infection.

On the other hand, arginine has an indirect effect on the IgA differentiation through the iNOS production. Indeed, the differentiation of B cells into IgA is mediated by iNOS (38). Therefore, the supplementation of arginine has been shown to increase the jejunal mucosal sIgA concentration in chickens under inflammatory conditions (36).

Amino Acids and Inflammation

During inflammation the plasmatic concentration of amino acids such as tryptophan, glutamine, proline, glycine, tyrosine, ornithine plasma decrease due to the increased demand of responding immune cells and liver for production of acute phase proteins (21). The redistribution of nutrients is a response of increased cytokine production such as interleukin-1 (IL-1), interleukin-1 (IL-6) and tumor necrosis factor (TNF-α) that are being released by immune cells. In addition, followed by infection, macrophages, produce nitric oxide (NO) from arginine by nitric oxide synthases (NOS) with the formation of citrulline. Inhibition of $NO^{\bullet}$ production increases the susceptibility to infection. Thus, arginine plays an essential role in the regulation of inflammation and immunity through $NO^{\bullet}$ synthesis, simulation of lymphocyte proliferation in Peyer's patches, modulation of T cells, induction of anti-inflammatory (IL-4 and IL-10) and suppression of pro-inflammatory (IFN- γ and IL2) cytokines (4). Arginine supplementation has shown to increase plasma levels of $NO^{\bullet}$ in chickens (12) as well as have a beneficial effect in the gut after a lipopolysaccharide (LPS) challenge (37). The positive effects are related with the reduction of expression of proinflammatory cytokines through the PPAR-γ activation (37).

Due to its oxidative capacity, arginine, cysteine, and glutamine are indispensable amino acids during inflammatory states. Antioxidants suppress inflammatory components produced during infection and stimulates cell mediated immunity (5). Sulfur amino acids including methionine and cystic are metabolized into glutathione (GSH), homocysteine and taurine, which play important roles in the intestinal immune response.

Cysteine (Cys) is converted into glutathione (GSH) which is the most important cellular antioxidant defense for the body (31). In chickens, cysteine is considered the limiting amino acid for GSH synthesis. A recent study showed that the supplementation of Cys decreased inflammation and oxidative stress under LPS challenge in weaned piglets (32). Specifically, the addition of 0.23 to 0.5 % of L-cys reduced the caspases-3 activity and the NF-kB. Since NF-kB enhances cell apoptosis, L-Cys may protect the intestinal permeability by regulating cell apoptosis and proliferation (32). Thus, cysteine is critical for maintaining oxidative stress in intestinal epithelial cells.

Besides being the major limiting AA for protein synthesis, methionine has been associated with intestinal function. The polyamide synthesis derived from methyl donation makes methionine an important biological antioxidant. Such functions indicated that methionine as a precursor for GSH synthesis is an effective nutritional intervention to improve gut mucosal function and to maintain epithelial cell survival and oxidative cell homeostasis (31).

Amino Acids and Necrotic Enteritis

The intestinal damage produced by necrotic enteritis starts with the colonization and degradation of intestinal mucus by its etiologic pathogen, *Clostridium perfringens* (CP) (28). In necrotic enteritis, the destruction of villi is a consequence of destruction of the lamina propria, extracellular matrix, intercellular junctions and attachment to epithelial cells (9). The binding of *Clostridium perfringens* to Toll-like receptors (TLRs) generates a cellular inflammatory response characterized by the differentiation of T helper cells into Th1, Th2, Th17, and Tregs cells (9). The activation of T cells result in the production of proinflammatory cytokines, including interleukin (IL)-1, interferon-γ (IFN- γ), IL-13, IL-17, (TGF)-β and IL-10 by Treg cells (6).

An increasing number of studies indicate the positive effect of the supplementation of dietary amino acids in preventing and treating inflammation associated with necrotic enteritis. Xue, et al., (2010) (45) reported that the prevalence of intestinal lesions from *Clostridium perfringens* infected broiler chickens were significantly reduced by supplementation of glutamine (10 g/kg). Indirect positive effects of Gln were observed by compensating the metabolic nutrient losses from lean muscle.

Likewise, the anti-inflammatory properties of arginine have proposed as a nutritional strategy for mitigating the impact of necrotic enteritis. An *in-vitro* study showed a bacteriostatic effect of NO$^{\bullet}$ derived from arginine against *Clostridium perfringens (*47*).* In addition, the supplementation of 1.87% of dietary L-arginine in broiler chickens challenged with a combined *Eimeria* and *Clostridium perfringens* significantly decreased the CP population in the caecum of challenged birds, improved the intestinal absorption, barrier function, and increased levels of anti-inflammatory (IFN-γ and IL-10) cytokines in the small intestine, ameliorating the adverse effects of necrotic enteritis (47). Moreover, a recent study showed that supplementation of 0.3% arginine reduced the incidence of NE lesions and restored the ileal microbial diversity and enriched microbial healing pathways that are significantly impacted during NE challenge (48). The mentioned studies indicated that the supplementation of dietary arginine could reduce the intestinal damage caused under necrotic enteritis challenges.

Another study reported that the supplementation of 0.2% and 0.4% dietary methionine (DL-Met) may also partially alleviate some of the negative effects of necrotic enteritis by lowering the numbers of *C. perfringens* in the ileum and cecum of NE challenged birds.

Amino Acids and Coccidiosis

Coccidiosis is one of the most important intestinal diseases worldwide. The pathogenicity of coccidia depends on the life cycle of the *Eimeria* inside the host (7). Because of the complexity of the life cycle of *Eimeria* the host immune response involves nonspecific (macrophages, granulocytes and natural killer), cellular (T lymphocytes) and humoral immunity (B lymphocytes, and IgA) (19). However, multiple studies have shown that T lymphocytes and the Th1-type cytokines (IFN γ and IL-10) mediated the anti-coccidial response against *Eimeria* infection (7). *Eimeria* oocysts infect the intestinal mucosa and produce enterocyte cell necrosis, inflammation, and atrophy of the villous (7). Nutritional strategies for controlling coccidiosis are focused on reducing oocyst shedding and modulating the T and B-specific cytokines in response to *Eimeria* infection through nutrient modifications.

The impact of threonine on the immunological response has been previously described. Zhang, et al., 2016 (48) reported that threonine deficiency increased the intestinal damage and oocyst shedding by 40% in coccidiosis challenged birds. The detrimental effect of the intestinal function is related with decreased IgA production within the ileal mucosa and a decrease in the T cell subpopulation (CD3), impairing the cytokine secretion profile of affected birds. Under pathological enteric conditions for which the threonine requirement is enhanced, the supplementation of threonine could be beneficial. A recent study showed that the supplementation of 5.3g/kg of threonine under coccidiosis challenge increased bird performance (42).

Other amino acids including arginine and glutamine are associated with alleviation of intestinal mucosal damage due to coccidiosis. According to (36) dietary supplementation of arginine reduced the expression of pro-inflammatory mediators via the suppression of the TLR4 pathway and increased the levels of mucosal maltase activity indicating enterocyte proliferation after infection. Another study reported a beneficial effect in gut morphology of coccidia-challenged broiler supplemented with 110% arginine (16) where villus height, villus height to crypt depth ration and villi surface area were significantly increased. Moreover, dietary manipulation of L-arginine for the control of coccidiosis has also targeted metabolic pathways for which L-arginine is a substrate, including nitric oxide (NO) synthesis by macrophage iNOS (1). Plasma concentrations of $NO^{\bullet}$ are related with the dose and virulence of *Eimeria* infection (18). (27) reported that supplementation of 0.03% of arginine in conjunction with vitamin E, decreased the concentration of NO in birds vaccinated against coccidiosis which resulted in a less severe inflammatory response against coccidia.

The supplementation of Gln has been recently proposed as a nutritional alternative to diminish the impact of live coccidiosis vaccines. Mussini, et al., (24) evaluated the effect of 0.5, 0.75 and 1% of Gln on broiler chickens vaccinated against coccidia. Results from this study, indicated that glutamine supplemented groups had significantly higher body weights at 21 and 28 days. The proposed mechanism of growth improvement is that supplementation of Gln will prevent muscle protein pull from the increase in demand for Gln for immune response and intestinal cell repair.

Conclusion

In addition to providing peptides for protein synthesis for growth development, amino acids have a great influence in intestinal functions. Numerous studies demonstrated that amino acids play an important role in maintaining intestinal cell development, intestinal epithelial barrier function and with regulating immune functions including intestinal inflammation. Glutamine provides a major energy source to enterocytes promoting cell proliferation. Molecules derived from arginine including nitric oxide, ornithine, and citrulline are directly involved in cell proliferation and immune response. Threonine is of great importance in maintaining barrier function and intestinal health and barrier function through mucin and IgA composition and by the stimulation of lymphocyte proliferation. Sulfur amino acids are important for enterocyte maintenance and functions, cysteine directly impacts the production of GSH, protecting epithelial cells from oxidation and promoting cell proliferation and survival.

The functions of amino acids in the reduction of intestinal inflammation can serve as a therapeutic nutritional intervention to ameliorate intestinal damage and to restore the impaired gut health of chickens under an enteric challenge.A better understanding of amino acid requirements of broilers under enteric challenges will allow nutritionists to model and predict dietary formulation based on enteric needs to improve nutrient efficiency and intestinal health.

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O[36] Current Potential and Limitations of the Low Protein Feeding Concept in Commercial Broiler Nutrition

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Feeding broilers with low protein diets supplemented with amino acids may reduce feed cost and allow using alternative feed ingredients. A significant part of the nitrogen intake is not retained by the broiler, but is excreted in the environment. High crude protein level compared with low crude protein level diets elevate the nitrogen and water excretion, which can reduce litter quality and may increase the incidence of footpad lesions. High crude protein level is also reported as a predisposing cause for necrotic enteritis. Therefore, it is important to investigate the possibility of lowering the crude protein levels using by-products and amino acids without impairing the birds' performance.

A floor pen trial was initiated by the Chamber of Agriculture, Lower Saxonia, Germany, and executed at the trial facilities of Haus Düsse, North-Rhine Westphalia, Germany. The objective of this trial was to investigate the effect of reduced crude protein levels on performance of ROSS 308 broilers in comparison to a common commercial diet.

Material and methods

In total 5000 one-day-old male and female broilers (Ross 308) were randomly distributed to four dietary treatments with an equal allocation of 250 birds into 20 floor pens. Each floor pen had a size of 17 m² (15 birds/m²), and was equipped with four bell feeders and 20 nipple drinkers for ad libitum supply of feed and water. Diets were formulated in line with breeder's recommendations for Ross 308 (Aviagen, 2014). The overview on the dietary treatments is given in Table 1.

Table 1: Overview on dietary treatments

Phase	Treatment 1 DLG-standard	Treatment 2 DLG N-reduced	Treatment 3 Medium N-reduced	Treatment 4 Strong N-reduced
	CP, %	CP, %	CP, %	CP, %
Starter (d1-10)	22.0	21.0	21.0	21.0
Grower I (d11-16)	20.6	20.0	19.5	19.0
Grower II (d17-30)	20.0	19.6	18.7	18.0
Finisher (d31-40)	19.5	18.9	18.0	17.0
Average of Treatment	20.4	19.5	18.7	17.9

Live performance, carcass evaluation, economic assessment, pododermatitis scoring (Hocking method 0 … 4), litter analysis on volume, dry matter, and nitrogen, as well as birds' nitrogen balance focusing on nitrogen excretions were investigated. Table 2 reveals results on live performance and the economic impact of the graded protein reduction.

Table 2: Results on live performance and economics

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Body weight (g)	2808[a]	2782[a]	2792[a]	2734[b]
Feed intake (g)	4615	4480	4433	4423
Feed per gain (g/g)	1.613	1.634	1.611	1.643
Mortality (%)	2.48	2.96	1.68	2.64
Feed per gain* (g/g)	1.593	1.609	1.597	1.620
European efficiency factor (EEF)	424[a]	413[ab]	426[a]	405[b]
Gross margin** (€/delivered bird)	0.884	0.863	0.884	0.812
Net profit***(€/delivered bird)	0.220	0.198	0.231	0.154

Different superscripts (a,b) indicate differences at $p<0.05$
* corrected for mortality by considering weight of lost birds
** Income over feed cost assuming 82 cent/kg bird weight, no statistics
*** considering cost of 10€/t litter and 287.5€/ other costs/1250 broilers (for all treatments), no statistics.

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The nitrogen excretion dropped following the reduction of diet's protein level (Table 3).

Table 3: Nitrogen balance & nitrogen excretion

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Average N-consumption (kg/pen)	35.19[a]	33.51[b]	32.52[c]	30.76[d]
Relative	*100*	*95*	*92*	*87*
Average N-retention (kg/pen)*	20.81[a]	20.56[ab]	20.77[a]	20.25[b]
Relative	*100*	*99*	*100*	*97*
Average N-utilization (% of intake)	58.4[d]	60.4[c]	63.3[b]	64.9[a]
Relative	*100*	*104*	*108*	*111*
Average N-excretion (g/place/year)**	439[d]	399[c]	355[b]	324[a]
Relative	*100*	*91*	*81*	*74*

Different superscripts (a-d indicate differences at $p<0.05$ * assuming 30 g N/kg body weight; inclusive bird losses
** 7.3 cycles per year

Further Benefits of the low protein could be proven by reduced litter volume (Figure 1), and an improved state of footpad health (Figure 2).

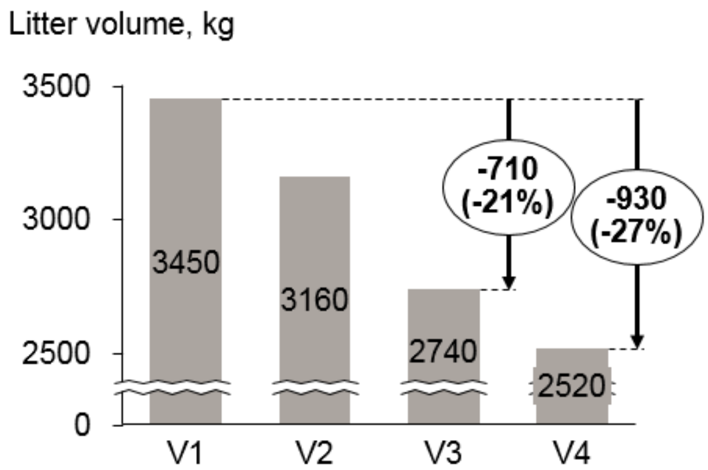

Figure 1: The impact of protein reduction on litter volume (kg)

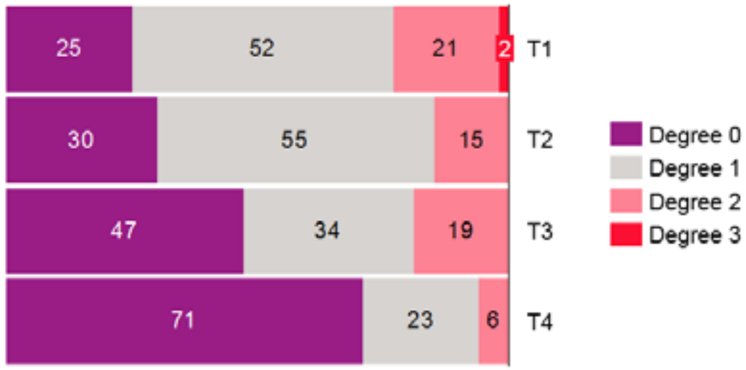

Figure 2: The effect of protein reduction on foot pad health*
*0 = no lesion, 1 = few lesions, 2 = medium, 3 = severe, 4 = very severe (not observed)

Summary and Conclusion

Crude protein reduction of about 1.5 % compared to standard broiler diets is feasible and practically applicable. A slight or no protein reduction is recommended for starter phase. Currently, glycine equivalence becomes the limiting factor for further implementation of the low protein concept in the field.

Feeding broilers with low protein diets supplemented with amino acids reduce feed cost and allow the usage of alternative feed ingredients.

Nitrogen excretion can be reduced effectively by lowering the crude protein concentration in feed. As a rule of thumb, also proven in this trial, the nitrogen excretion dropped by about 10 % per 1 % CP reduction.

O37 The Role of Oregano Essential Oil in Promoting Broiler Growth Performance under *Eimeria* Challenge

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Introduction

Coccidiosis and necrotic enteritis can compromise gut health and overall performance of birds, resulting in poor feed conversion ratios, reduced growth performance and increased mortality[5]. These diseases are estimated to cost the global poultry industry $2-3 billion per annum and up to $0.05/bird respectively [5]. Challenges to gut health can be further intensified as a result of the pressure to reduce or remove antibiotics and anti-coccidials utilised in either a therapeutic or prophylactic manner to defend against such protozoa [2]. Therefore, finding an alternative to antibiotics and anti-coccidials for broilers, whilst maintaining coccidial recycling in poultry litter in order to ensure development of natural coccidial immunity, is of the upmost importance in both achieving and supporting bird liveability, health and performance.

The use of oregano essential oil (OEO) in broiler diets has been well documented to have beneficial properties, including acting as an anti-coccidial[3], immunomodulator and antioxidant, ultimately improving gut health and therefore resulting in the achievement of enhanced performance parameters [4]. Therefore, the use of oregano may be a suitable alternative to in-feed anti-coccidials, such as robenidine hydrochloride.

Materials and Methods

3080 Cobb 500 male broiler chicks were randomly allocated at day old to 1 of 7 treatments, with 8 replicates of 55 birds per treatment (a further 40 chicks were reared on basal diet for the coccidial immunity check).

Starter feed was fed from day 0 to 21. On day 21, non-consumed starter feed was weighed and discarded. Grower feed was then fed until day 35 when non-consumed feed was weighed and discarded. Finisher feed was fed until day 42 with on-consumed feed weighed and discarded. All treatments included a standard commercial dietary ration and were fed through all feeding stages, containing either treatment: 1) ROB – anti-coccidial robenidine hydrochloride (Robenz, Elanco, USA) at 33 mg/kg; Oregano essential oil (Orego-Stim®, Anpario plc, UK) was included in feed at either; 2) OEO (150g/t); 3) OEO (300g/t); 4) OEO (450g/t); or Oregano essential oil (Orego-Stim® Liquid, Anpario plc, UK) was added to the water at either 5) OEO (75ml) – 75ml/1000L; 6) OEO (150ml) – 150ml/1000L; or 7) OEO (300ml) – 300ml/1000L. All birds were vaccinated with an attenuated *Eimeria* vaccine at the hatchery (HatchPac Cocci III (Merial, USA)) on day 0.

Bird weights by pen were taken on days 0, 21, 28, 35 and 42. On day 21, 5 birds from each pen were removed and challenged orally with a combination of *E. acervulina*, *E. maxima* and *E. tenella* (in a ratio of 3:2:1 sporulated oocysts/bird). Six days post-challenge, these birds were euthanised and coccidial lesion scored for degree of infection of *E. acervulina*, *E. maxima* and *E. tenella,* The system of Johnson and Reid (1970) was used, wherein 0 is normal and 4 is severe infection. A group made up of 40 non-vaccinated coccia-free birds was used as a positive control (POS). On days 0, 7, 14, 21, 28, 35 and 42 fresh faecal samples were taken from each pen and tested for coccidial oocysts shedding per gram (OPG).

Data was analyzed via ANOVA (JMP v13.2.1 SAS Institute Inc. USA) using pen means and significance was detected at $p<0.05$.

Results and Discussion

Feed intake and body weight gain was similar across treatments except between ROB and OS (150ml) (2.138 and 2.228kg body weight gain and 176.66 and 185.11g/day respectively $p<0.05$. Table 1). There was no difference in weight adjusted feed conversion ratio (FCR) across all treatments.

OEO allowed the presence of a low-level cycling of coccidia in the litter, without clinical symptoms of coccidiosis being present, which provides an oocyst population enabling active immunity to develop within the birds (Table 2).

Following the immunity challenge lesion scores were highest in the POS CON treatment group, and significantly reduced in the ROB group versus all other treatments (Table 3). OEO treatment groups performed, in general, similar to the anti-coccidial group but OS (300g), OS (450g) and OS (150ml) showed significantly lower average lesion scores compared to the ROB treatment and POS CON (1.0, 1.1, 1.1, vs 1.5 and 2.8 respectively). Weight gain was greater in birds fed OEO 150g), OS (300g), OS (450g), OS (150ml) and OS (300ml) treatment groups versus ROB. Weight gain was greater in all OEO treatment groups compared with POS CON.

Table 1. Growth performance parameters (0-42 days) of birds treated with in-feed anti-coccidial or with oregano essential oils (OEO).

Treatments	Feed Intake (g/day)	Adj. FCR (kg/kg)	Wt Gain (kg)
ROB	176.66b	1.646	2.138b
OS(150g)	181.74ab	1.678	2.141b
OS(300g)	179.35ab	1.664	2.151ab
OS(450g)	177.49ab	1.658	2.175ab
OS(75ml)	184.00ab	1.678	2.172ab
OS(150ml)	185.11a	1.665	2.228a
OS(300ml)	184.17ab	1.676	2.179ab

a-b letters that differ within a column denote significant differences at $p<0.05$

Table 2. Weekly coccidia count (Oocysts (10^3)/g) in litter of birds treated with in-feed anti-coccidial or with oregano essential oils (OEO).

Treatments	ROB	OS(150g)	OS(300g)	OS(450g)	OS(75ml)	OS(150ml)	OS(300ml)
Day 7	0.05c	6.48a	4.22ac	3.77ab	2.41bc	3.02bc	1.06bc
Day 14	0.75c	11.56a	11.31ab	5.08b	4.42c	3.02b	2.36c
Day 21	1.21b	10.55a	10.05a	5.78ab	7.24ab	5.48ab	3.92ab
Day 28	1.06a	3.92a	3.12a	2.06a	3.52a	4.98a	1.51a
Day 35	1.61a	0.75a	0.85a	0.65a	0.80a	1.11a	1.56a

a-c letters that differ within a row denote significant differences at $p<0.05$

Table 3. Immunity evaluation following combined *Eimeria* challenge on day 27 (6 days post challenge)

Treatments	Average Lesion score	Weight gain (kg)
ROB	1.5b	0.157b
OS(150g)	1.2bc	0.227a
OS(300g)	1.0c	0.215a
OS(450g)	1.1c	0.222a
OS(75ml)	1.3bc	0.205ab
OS(150ml)	1.1c	0.214a
OS(300ml)	1.3bc	0.213a
POS CON	2.8a	0.082c

a-c letters that differ within a column denote significant differences at $p<0.05$

Conclusion

OEO included in the diet of poultry has been shown to maintain weight gain and feed efficiency and allow development of natural coccidial immunity through coccidial recycling within the litter to the same extent achieved with anti-coccidials such as robenidine hydrochloride. Following immune challenge with *Eimeria* spp. lesion scores were significantly reduced in 300g, 450g and 150ml OEO treatment groups and this had a beneficial effect on body weight gain, providing a significant benefit over robenidine hydrochloride. Therefore, the supplementation of broiler diets with OEO may have a beneficial effect in the prevention of coccidiosis and necrotic enteritis whilst reducing the need for antibiotic coccidiostats, producing a resilient bird for modern broiler production systems.

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O38 Effects of Guanidino Acetic Acid Supplementation to Broiler Diets Having Reduced Energy on Performance and Carcass Quality

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Introduction

Energy is a component of poultry feeds; feed costs cover 70% of all broiler production costs and about half of this 70% share is related to the energy content of the feed so in broiler production representing about half of the expenses that producers have to pay for broilers' feed (1). Current feeding strategies for poultry include greater accuracy in nutrient provision to not only support optimal growth performance but also reduce input costs and the environmental impact associated with feeding excess nutrients. As such, nutritionists are always looking for novel approaches to improve availability of energy for broiler chickens. One mechanism to store the flow of energy in the body within the cells is mediated through the formation of high-energy phosphate bonds (2).

Guanidinoacetic acid (GAA), also referred to as glycoamine or guanidinoacetate, is a natural precursor of creatine in the vertebrate body. GAA is formed from the amino acids glycine and arginine in the kidney and absorbed from the gut. It is subsequently methyllated by S-adenosylmethione and transformed to creatine in the liver. All the processes of cells involved in growth and metabolism require energy. In body cells, adenosine triphosphate (ATP), which is the main source of energy, is used for maintenance and growth. Phosphocreatine serves as a dynamic reservoir of high energy phosphate for ATP formation (3,4,5). In general, about 1.5- 2 % of the creatine and phosphocreatine pool is irreversibly converted to creatinine each day and excreted in the urine (6). Therefore, creatine must be continually replaced. However, the amount that is synthesized could be insufficient to meet the demand of the fast growing broilers. Based on data from human research on vegetarians (7) but also on experience after the ban of animal by-products in broiler nutrition, when poultry performance (especially feed conversion) was impaired (8), it is assumed that the potential of the de-novo synthesis for optimum creatine supply is limited. Creatine can thus be seen as a semi-essential substance. The animal's demand for creatine can be supplied either directly from animal protein (e.g., fish or animal by-product meal) in the diet or by endogenous synthesis. Creatine and GAA are not found in plants and consequently under pure vegetable feeding conditions all creatine must be derived by de-novo synthesis. On behalf of this GAA has the potential for being supplemented to broilers since it plays an important role in the cellular energy metabolism (2).

Therefore, in the present study, the objective was to evaluate the efficacy of supplementing GAA (supplemented as CreAMINO®-containing a minimum of 96% GAA-) to a typical corn soybean meal diets with graded levels of ME on performance and carcass characteristics of broiler chickens form 0 to 41 days of age.

Material and Methods

Birds and Housing

The research was carried out in broiler research house of Beypilic Broiler Company, one of the largest broiler integration, in Bolu province, Turkey. 10260 one-day-old, mixed sex (5320 male, 5320 female) Ross-308 chicks were weighed and randomly allocated to 54 floor pens (6.5 × 2 m). Rearing conditions were set according to breeder guidelines (Anonymous 2014). Chicks were allowed ad libitum access to feed and water. The research was conducted according to a completely randomized 3*2 factorial design. Day old chicks were weighed and randomly distributed into 6 dietary treatments each has 9 replicates with 190 chicks in floor pens of experiment house through 41 days. Three phase feeding program was administered through the experiment as starter (0-11 days), grower (12-25 days) and finisher (26-41 days).

Diets

Diets for each feeding period were formulated to meet or exceed breeder guidelines (9), but with different levels of metabolic energy (ME) as given in Table 1. Treatments consisted 3 different energy level (basal - 50 kcal/kg low – 100 kcal/kg low) and guanidino acetic acid supplement (%0.06) or no supplement (% 0.00). 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 in Beypiliç Feed Mill.

Table 1: Experimental design

Treatments	**Treatment Code**	**GAA and Energy Difference in the Treatments**
Positive Control	PC	Control
Negative Control	NC50	Control -50 kcal/kg ME
Negative Control-2	NC100	Control -100 kcal/kg ME
Negative Control+GAA	PC+GAA	Control + %0.06 GAA
Negative Control-1 +GAA	NC50+GAA	Control -50 kcal/kg ME + %0.06 GAA
Negative Control-2 +GAA	NC100+GAA	Control -100 kcal/kg ME + %0.06 GAA

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.

Statistical Analysis

The data were analyzed as a completely randomized block design with 6 dietary treatments and 9 replicates using the ANOVA/MANOVA procedure of the MINITAB (Statistical Software, version 13). The main effects and interactions between main effects were analyzed and when significant differences ($P<0.05$) among groups were found, means were separated using the Tukey HSD test.

Results

Performance and carcass quality

The effects of GAA supplementation and metabolic energy level on final body weight and whole period FCR were evaluated. According to the final performance data, while GAA supplementation significantly improved final body weight, energy level did not affect final body weight. Similarly, GAA supplementation significantly improved whole period FCR but energy reduction significantly increased whole period FCR. NC50 and NC100 -both energy reduction treatments- significantly increased FCR compared to basal –normal energy level- diet. While feed intake was not affected by GAA supplementation, 100 kcal/kg energy reduction significantly increased feed intake compared to basal diet. Mortality was not affected by either GAA supplementation or energy reduction. Similarly, carcass parameters (carcass yield, leg meat yield, breast meat yield and wing meat yield) were not affected by GAA supplementation or energy reduction except for 100 kcal/kg reduction significantly decreased wing meat yield compared to basal diet.

Discussion

Performance results showed that, feed consumption is affected by the energy level and it is indicating that chicks consumed more feed to compensate energy requirement and remove performance. In this study, while chicks consumed energy deficient feed were only decreased FCR, final body weight was not affected. In literature it is mentioned that, the effects of the differences in feed energy on the FCR are expected to be more intense than the effects on body weight gain (10, 11, 12). This result verifying other studies (13-14) indicating that energy reduction has negative effects on FCR.

As mentioned before, one of the key factors in cellular energy metabolism is creatinine. Creatine phosphate is an important reserve that can be mobilized quickly in high-energy phosphate to be used for ATP formation. Especially creatine needs are important in fast growing animals (14). In that study GAA supplementation improved final body weight and FCR. So present study shows the positive effects of GAA on the growth performance of broiler chicks by contributing to the energy metabolism of the creatinine precursor. In line with this study other study mentioned that GAA or creatine supplementation were improved FCR and performance (2, 13, 14, 15, 16).

Carcass parameters showed that, GAA supplementation was not affected carcass yield, leg meat yield, breast meat yield and wing meat yield. Our results supported by the findings of studies that GAA supplementation was not effects on carcass parameters (13, 14, 15, 16).

Conclusion

Considering the growth performance and carcass parameters it might be concluded that on top GAA supplementation at level of 0,6 g/kg (%0.06) may improve final body weight and feed conversion ratio (FCR), possibly by affecting energy metabolism positively and it is possible to save 50 kcal /kg ME without any adverse effect on performance and carcass quality.

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IS[17] Current Respiratory Diseases Problems of Broilers and Vaccination Strategies

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Abstract

The poultry sector is one of the fast growing and the most dynamic segments of the livestock sector worldwide. It is estimated that average poultry meat consumption in the world will increase by 3.7 kg in 2030 and approximately 40% of the meat consumed will be poultry meat. The poultry sector in our country is of great importance in terms of feeding our people and our national economy.

The pathogens that existed in the field have gained the ability to spread more rapidly by switching to intensive production in parallel with the development of the poultry industry.

According to the data of the Ministry of Agriculture and Forestry, Poultry Database, 2018, there are 319 broiler breeder farms, 2,180 broiler breeder flocks, 22,446,451 broiler breeder capacity, 7,655 commercial broiler farms, 12,542 broiler flocks and 267,178,862 broilers.
In Turkey the respiratory system problems constitute important economic losses and emerge as a problem. In this review, the important respiratory system agents and problems they cause problems in broiler and breeding flocks in different regions of Turkey is to be discussed.

Key Words: Newcastle Diseases, H9N2, Infectious Bronchitis, Infectious Laryngotracheitis, Vaccination

O39 A Retrospective Evaluation of the Prevalence of NDV in Provinces Affiliated to the Directorate of Samsun Veterinary Control Institute

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Abstract

Newcastle disease (NDV) is an acute, rapidly spreading, infectious and lethal viral disease of poultry. The cause of Newcastle disease is included in the paramyxovirus species (PMV-1) of Myxoviruses. There are 9 serogroups identified in paramyxoviruses. “Group 1” is the most important of these and Newcastle disease virus (PMV-1) is located in this serogroup. Infection affects the respiratory, digestive and nervous systems of poultry. In this study; it is aimed to evaluate the prevalence of NDV in morbid substances retrospectively which sent from provinces affiliated to the Directorate of Samsun Veterinary Control Institute between 2014-2018.

The diagnosis of NDV from morbid substances was carried out by the methods described in the Diagnostic Method Union book for diagnosis of poultry diseases by the Ministry of Agriculture and Forestry, hemagglutination inhibition test and virus fusion gene target Real-Time PCR.

As a result of the retrospective evaluation of the results, 121 (24.59%) of 492 samples, which sent from provinces affiliated to the Directorate of Samsun Veterinary Control Institute between 2014-2018 from 73 outbreak points and reported as disease area [Amasya (0), Giresun (3), Ordu (6) Rize (4), Samsun (42), Sinop (2), Sivas (3), Tokat (4), Trabzon (9)], were detected as positive. The evaluation of the results according to years, the positivity rates between 2014-2018 were determined as 16.66%, 21.17%, 30.0%, 16.87% and 36.30%, respectively. The evaluation at the level of affiliated provinces, the positivity rates in Amasya, Giresun, Ordu, Rize, Samsun, Sinop, Sivas, Tokat and Trabzon were 0%, 38.46%, 41.17%, 13.55%, 26.01%, 16.0%, 22.22%, 16.32 and 47.5%, respectively. When the findings were examined, it was seen that the province with the most positivity was Trabzon and the year with the highest number of positives in all provinces was 2018.

Consequently, NDV continues to be detected in our region as well as in Türkiye and in order to reduce the prevalence, the necessity of increasing the protection and control measures has been demonstrated.

O40 Molecular Characterization and Phylogenetic Analysis of Marek's disease virus *Meq* Gene in Turkish Poultry Flocks

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Abstract

Despite vaccination, Marek's disease (MD) still seen in many countries and is usually associated with changes in virulence, improper vaccination and biosecurity. Therefore, it is important to analyse Marek's disease virus (MDV) *Meq* gene sequences by phylogentic analysis. Phylogeny of the MDV *Meq* gene has been studied in many countries. However, there is no data about the genotypes and phylogeny of Marek's disease virus circulating in Turkish poultry flocks. Hence, this study was performed to sequence MDV *Meq* gene and phylogenetic analyses. For this, in 2017 and beginning of 2018, a total of 403 spleen samples from breeder and layer flocks were collected from the Marmara, West Black sea and Aegean regions. DNA was extracted from the spleen samples and the samples were analyzed by PCR by using primers targeted to MDV *Meq* gene. After sequencing, phylogenetic analysis was performed to compare the similarity and diversity of the strains reported to Gene Bank from other countries. Amongst 33 flocks analyzed, virulent MDV was detected in 6 flocks. Splenomegali, hepatomegali and tumours in the oviduct were observed in chickens of affected flocks. Virulent MDV was detected in 97 of 403 spleen samples analyzed. Sequencing and phylogenetic analyses have shown that MDVs detected in this study were similar to MDV strain from Poland and closely related to MDV strain from Saudi Arabia.

In conclusion, this is the first study on the phylogenetic analyses of MDV *Meq* gene in layers and breeders in Turkey. The results have shown that MDV still effects poultry health and the phylogenetic data obtained here will help in vaccination and control strategies.

Key words: Marek Disease virus, Chicken, PCR, *Meq* gene, sequencing, phylogenetic

IS[17] Leg Health Problems In Broilers Related To Hatchery

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Abstract

Commercial meat chicken life spans continue to decrease with the regular improvements provided by genetic selection in this type of bird. Incubation time for fertile broiler eggs however has not changed and as a result, the embryo's time in the egg now represents over 37% of the bird's commercial life. Factors involved in incubation may therefore be contributing to a greater proportion of the broiler's development and health. The changes in the broiler's genotype may also have affected its incubation behaviour and there are known differences in the response of each of the major broiler strains used worldwide during their time in the incubator. The Cobb strain has been shown to produce more heat and to hatch much earlier than the Ross strain under the same incubation conditions. Many studies have indicated possible influence of varied incubation conditions, particularly temperature, on subsequent chick quality, growth performance and musculo-skeletal integrity. Our laboratory has conducted a series of experiments looking at the latter issue with a focus on bone mineralization at hatch/take-off and subsequent leg strength after grow out. Summarized results are reported from three experiments in this series. In all experiments, eggs were incubated at the identified standard "ideal" incubation temperature (measured by eggshell temperature - EST) of 37.8^{o}C throughout the setter period and a program involving starting with a slightly lower EST with a gradual increase up to 37.8^{o}C by 16 days of incubation (termed "Slow start"). At hatch or take-off time, some chicks were sampled for serum Ca and P, chick weight and length and femoral bone ash determination. Remaining birds were then grown out to 35 or 42 days and males were subjected to a modified Latency-to-Lie test to estimate leg strength.

The Slow start incubation temperature profile delayed the hatch time of the Cobb strain to the same as the Ross strain under standard incubation conditions. Early hatching chicks have a longer sojourn in the hatcher as a chick, outside the egg. Early hatched chicks continue to grow (bone length increases) even without food during this sojourn; utilizing their yolk reserves. However this results generally in a decline in skeletal mineralization, possibly as the chick reaches a limitation of phosphorus reserve in the yolk. Delaying Cobb chick hatching time and thus reducing this sojourn, results in a chick with higher bone ash at take-off. This also deceases some of the late incubation embryo mortality. The Slow start profile applied to the Ross strain is however deleterious to its hatchability and leads to a decline in bone ash at take-off. The higher bone mineralization of the chick can translate to better ability to stand in the Latency-to-Lie test in males at 35 days of age.

The importance of considering the strain of broiler used in incubation studies is strongly highlighted.

Introduction

Leg weakness in broiler chickens has been a considerable but varied problem for the meat chicken industry over many years. Genetic progress with the meat chicken continues to increase growth rate and improve feed use efficiency. As a response to concerns about skeletal health, breeder companies have responded by attempting selection aimed at improved leg strength and this has been largely successful. Both major international commercial broiler breeds now exhibit improved leg strength and, combined with a shorter growing period, leg weakness issues are continually declining. Broilers now reach market weight by 30-35 days while egg incubation times have not changed. Hence incubation now accounts for over 37% of the broiler's entire lifespan. The events experienced by the developing embryo thus have an ever increasing influence on its remaining life. Genotype improvements affecting the hatched chick's growth and development also have effects on the metabolism of the bird while it is an embryo [1].

Influence of incubation factors have long been known to contribute to chick quality and affect subsequent growth and performance [2].

Our laboratory has been conducting investigations into the associations between incubation factors and subsequent broiler leg strength for some time now. In Australia, the Cobb broiler had a reputation for poorer leg strength and we see out to look at any effect differing incubation conditions had on this breed. However the breeders of the Cobb in Australia made rapid genetic and management improvements, which resulted in each subsequent incubation experiment that we conducted was using a different genotype. Changes made to the bird over time included a change from feather-sexable to non-sexable broilers, an introduction of a new sire line and changes in the selection method employed at great-grandparent level. This made the ability to make reproducible experimental rather complicated. In addition, the experimental variables examined focused on incubation, but assessment of leg strength was most reasonably done at 5 weeks of age, as leg issues develop as the bird reaches higher weights [3,4]. Obviously there are a variety of other factors which can influence leg strength during growth that are not related to incubation (e.g. growth rate, nutrition, infective causes, litter conditions) and variability in these from trial to trial also results in variable outcomes. Under this scenario, development of reliable conclusions about incubation contributions to the final outcome of leg weakness involved conducting meta-analyses on our data across several similar experiments to overcome some of this extraneous variability. Using this statistical approach [5], we were able to demonstrate that what was regarded as the "ideal" incubation temperature for broiler eggs [6] did not produce the best results in terms of skeletal parameters in the Cobb broilers which we studied. Others have also described marked differences in embryonic growth in embryos of the two major broiler breeds [7], with the Cobb bird incubating more quickly than the Ross. In our experience, if incubated at the "ideal" egg shell temperature of 37.8°C [6], the Cobb broiler hatched up to 18-24 hours earlier then the Ross [8]. This earlier hatching time can affect bone mineralization when chicks are removed from the hatcher and this can lead to problems with leg strength and bird locomotion later in life [5,H]. This lead to a variation in incubation temperature profiles being studied [9] which showed differences in bone mineralization at hatch and subsequent variations in leg strength and ability of the bird to stand for a time at 5-6 weeks of age. An overview of the experimental findings in this project will be presented using results from three experiments.

Materials and Methods

All experimental procedures were supervised by the Animal Ethics Committee of the University of Sydney. All experiments used small E2A incubators (Multiquip Pty Ltd, Austral, NSW, Australia), which have forced ventilation, evaporative humidity, digital temperature control and automated turning for the setting period (ED1-18). Egg shell temperatures (EST) were measured in all experiments continuously in real time using sensors applied to individual eggs during days 1 to 18 of incubation (ED1 to ED18). For hatching, all eggs were transferred into one Aussieset™ incubator (Bellsouth Pty Limited, Narre Warren, Victoria, Australia) for the hatching period, which terminated at 516 h (21.5 days) of incubation. Remote Intelligence sensors (Netic Pty Limited, Ryde, NSW, Australia) were kept in contact with the equator of eggs in each tray in each incubator with connection to a remote monitor. The real time readout was used to adjust the incubators to maintain the EST desired for each experiment. Chicks were removed from the hatcher at a standardized time of 516 h (21.5 d) of total incubation time. The chicks hatched in individual cells in the hatcher trays allowing identification of which egg produced each chick. Hatching was monitored 6-hourly from 468 h of incubation until take-off of the chicks. At take-off a sample of chicks were compared using chick length, chick weight, serum Ca and P and femoral bone ash (BA). The remaining chicks wee grown out to 5-6 weeks and leg strength in males was compared using a modified Latency-to-Lie test (LTL). LTL involved standing the bird in a tub with about a 3cm depth of tepid water and measuring the time that the bird took to sit down, up to a maximum of 5 minutes.

The experiments considered in this report followed the following designs
Experiment 1 incubated Cobb eggs at either the "Standard" EST of 37.8^{o}C throughout ED 1-18 compared to a lower starting EST of 37.3^{o}C at ED 1 rising progressively to 37.8^{o}C by ED 16 and then maintained. The latter profile was termed "Slow start" profile.

In Experiment 2, Cobb eggs were incubated using a slow start profile which began at a lower starting EST of 36.9^{o}C and again rose to 37.8^{o}C by ED 16 compared to the standard profile. In this experiment, some chicks which hatched early (<492 h of incubation) were removed early and sampled or fed immediately. These were compared to chicks that hatched early and remained in the hatcher until 516 h and those which hatched late (>492 h).
Experiment 3 compared Cobb and Ross chicks which were subjected to either the standard or slow incubation profiles.

Results

Unexpectedly but satisfyingly, hatchability was improved in the Cobb eggs by the slow start incubation profile in all experiments.

The "Slow start" incubation profile in Experiment 1 delayed mean hatch time for the Cobb broiler by 9 hours (Figure 1).

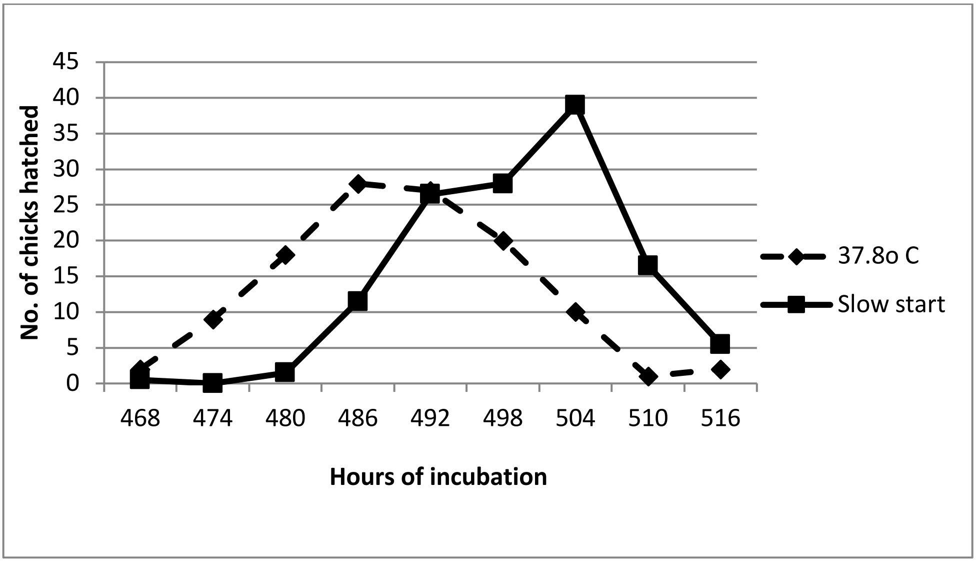

Figure 1. Hatch time spread of chicks from Cobb eggs incubated using a Standard profile (37.8°C) and a “Slow start” profile (37.3°C at ED1 rising to 37.8°C by ED 16). Mean hatch time for the standard profile was 490 h but the Slow start profile significantly extended mean hatch time to 499 h (P= 0.00008).

At the time of chick take-off (516 h of incubation) the earlier hatching chicks were lower in bodyweight than the slow start chicks (38.6g compared to 41.4g respectively) which was due to a difference in retained yolk weight. But they were slightly longer (18.8cm versus 18.6cm). The earlier hatching chicks had continued to grow after hatching, using up yolk reserves. As they were longer, bone growth had also continued. Effects were seen in association with the length of time the chick spent outside the egg after hatching in the hatcher (called its “sojourn”). Longer sojourn was correlated with a decline in total chick weight, an increase in chick length and a consequent decline in yolk weight. Also interestingly, serum Ca increased while serum P decreased with a longer sojourn. For the chicks that were grown on, the earlier hatched group gained significantly more weight in the first 7 days than the slow start group (122 g gain compared to 117 g). These findings were to be consistent with all the studies. This faster early growth may have reflected greater hunger in those chicks with a long sojourn, as feed intake on the first day was significantly increased. The weight advantage however was not maintained to the end of growth. The effect in hatch time delay was not thought to be fully effective as required as the desired mean hatch time should be closer to 504 h incubation. Hence the early EST targets for the next experiment was reduced to 36.9°C, rising gradually to 37.8°C by 16ED.

The reduced EST at the start for the Slow incubation group increased time of hatch by 11 h (mean of 503 h compared to 491h for the Standard incubation profile). In this experiment, some early hatched chicks (hatched <492 h) were removed from the hatcher at 492 h and sampled (group EE) and some were immediately placed on feed (group EF) and sampled at 516 h from the start of incubation. Some of the early hatchlings remained in the hatcher for the longer sojourn (group EL). Later hatching chicks hatched (>492 h; group LL) were also compared to these other groups at 516 h after the start of incubation. At 516 h after the start of incubation LL chicks weights were similar to EE chicks at time of hatch but were greater than for those that hatched early but were kept in the incubator for 24 hours (EL). Not surprisingly, early hatched chicks that were fed for 24 hours (EF) weighed significantly more than the other groups. LL chicks were the shortest and we did not show an increase in length of the EL chicks over the EE starting length in this experiment.

Later hatching chicks (LL) had significantly higher femoral bone ash (BA) than those that hatched early (EE and EL), while EF (fed) chicks had even higher BA (but not significantly different to LL at the same time).

Table 1 shows sample results from chicks in each group. Early hatched chicks with a long sojourn (EL) revealed a decrease in yolk weight and a decline in the total amount of Ca and P stored in the residual yolk compared with chicks hatched at a similar time but sampled at 492 h (EE). EL chicks had an increased serum Ca and a reduced serum P compared to EE chicks. Chicks that hatched late (LL) also had a lower serum P and residual yolk Ca and P than EE (but higher yolk reserves of P than EL chicks), indicating continued use of these minerals during their longer time to hatch. Birds that hatched early and were immediately fed (EF) gained weight (about 7g between 492 h and 516 h after the start of incubation) but also had higher serum Ca and P, similar yolk reserves of P to LL but an increased amount of Ca in the yolk.

Table 1. Experiment 2: results from chicks sampled at 516 hours after the start of incubation

Group	n	Serum Ca mmol/L	Serum P mmol/L	Yolk Wt gm	Total yolk Ca mg	Total yolk P mg	Femur Ash % Hatch
EE	39	2.34^{C}	1.46^{A}	5.67^{A}	2.248^{A}	0.105^{A}	22.6^{B}
EL	41	2.46^{B}	1.22^{C}	3.34^{C}	1.742^{B}	0.062^{C}	23.7^{B}
LL	38	2.40^{C}	1.21^{C}	4.74^{B}	1.800^{B}	0.080^{B}	24.6^{A}
EF	37	2.76^{A}	1.31^{BC}	2.96^{C}	2.404^{A}	0.079^{B}	26.0^{A}
P=		*<0.001*	*0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.0001*

ABC Means with different superscripts differ (P<0.05)
EE – chicks hatched before 492 hours and taken off at 492 hours; EL – chicks hatched before 492 hours but remaining in the incubator until 516 hours; LL – chicks hatched after 492 hours and taken off at 516 hours; and EF – chicks hatched before 492 hours, taken off at 492 hours and placed on feed.

Interestingly, early hatched chicks which remained in the hatcher (EL) had a faster growth rate in the first 7 days than either the late hatched chicks (LL) or the early hatched chicks which were fed early (EF) and the latter groups were identical in 7 day weight (EL 133g, LL 126g and EF 125g). This experiment was affected by a coccidiosis incidence late in rearing and growth rate was reduced. As a result LTL results were high for all groups (mean LTL was 133s for EL, 208s for LL and 266s for EF) but the early fed group stood for significantly longer in LTL than either of the other groups (P=0.03).

Results from Experiment 3 showed marked differences in the response of the two broiler strains to varied incubation profiles. On the standard incubation profile mean hatch time for Cobb was

484 h but for Ross it was 493 h. Exposing both strains to the slower profile extended hatch times for both by 13-14 h. The Slow incubation profile increased the overall hatch rate for the Cobb bird (as seen in the other experiments) but this slower incubation profile decreased hatch for Ross. In both strains the phenomenon of faster growth by day 7 for earlier hatchlings with a longer sojourn was observed. A strain interaction with bone mineralization and hatch time was noted. While overall mean BA was significantly higher for the Ross chicks compared to Cobb, BA for the Cobb bird increased when the slow incubation profile was used, whereas it decreased for the Ross bird under these conditions (Figure 2).

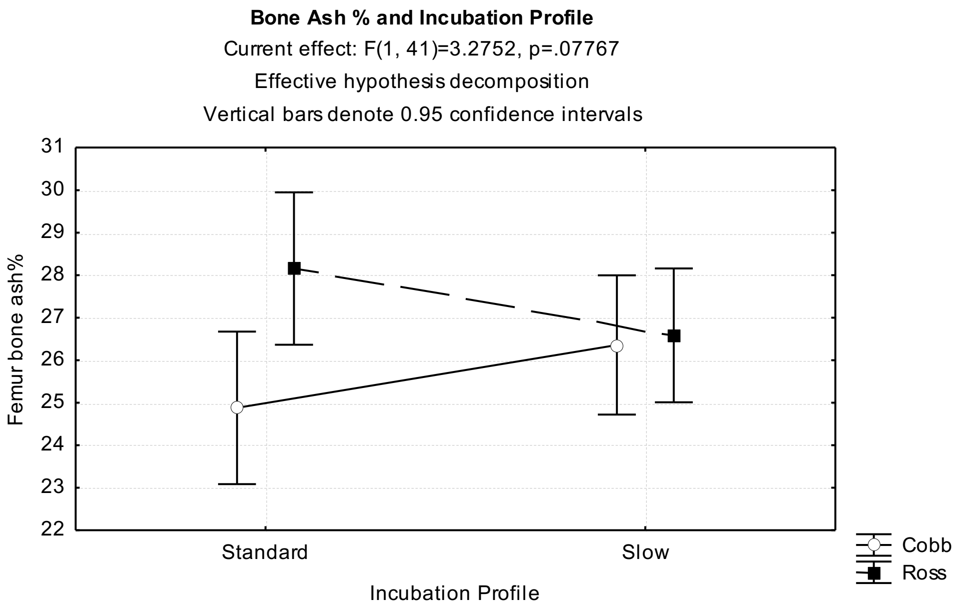

Figure 2. Interaction of broiler strain with femoral bone ash at take-off between standard and slow incubation profiles. .

Interestingly, the slow incubation profile led to better leg strength in both broiler strains, as shown by significantly longer LTL results for males at 35 days of age (Table 2). There was also no difference between the strains in LTL.

Table 2. Latency-to-Lie[1] tests comparing slow[2] and standard[3] incubation profiles in Cobb and Ross broilers

Broiler strain	Incubation profile	Median LTL[1] (seconds)
Ross	Slow[2]	161^{A}
	Standard[3]	106^{B}
Cobb	Slow	171^{A}
	Standard	113^{B}
Gehan's Wilcoxon Test	*P=*	*0.03*

^{A,B} Medians without common superscripts differ (P<0.05)
[1] Latency-to-Lie – time remaining standing
[2] Slow start incubation (EST beginning at 36.9ºC and rising to 37.8ºC by 16 days)
[3] Standard incubation (EST 37.8ºC throughout)

Discussion

Many researchers have reported deleterious effects from altering incubation (eggshell) temperature from the accepted "ideal" of 37.8°C [10-13]. It is important when assessing the literature on this issue that the strain of broiler is considered. Many of these studies have used the Ross strain and we and others have shown that incubation behaviour differs markedly between Ross and Cobb strains [7,14]. Reported conflicts of findings in the literature can also be related to the strain of broiler studied e.g. [11] compared to [12].

It is clear that altering the incubation temperature profile for the Cobb strain, based on EST, results in improved outcomes for that broiler. Hatchability is improved, bone mineralization at take-off is improved and this has been shown to correlate with better leg strength later in the grow out [5], which was clearly seen in Experiment 3 in the present series.

Higher eggshell temperatures (i.e. compared to the "ideal") in the first two-thirds of incubation will lead to earlier hatching, whereas a lower EST in this period delays hatching [5]. We contend however that the ideal EST for Cobb broiler eggs should be below this established ideal of 37.8°C, and as such, use of the accepted "ideal" EST in this strain equates to excessive temperature and results in early hatching, with the deleterious effects described by many [10-13] as a result.

Results from Experiment 2 demonstrated that bone mineralization of Cobb hatchlings can be improved by delaying incubation period to a more normal time (504 h), or by removing the chicks from the hatcher earlier and supplying feed [9].

Locomotory ability and ability to stand for longer at 35 days of age is strongly correlated with fast growth and variations in growth rate can affect these types of experiments. Bone strength is not the only factor that can be affected, as muscle development has also been shown to be adversely affected by high EST [15].

An issue which may bear on the bone strength of newly hatched chicks may relate to the amount of minerals present in the yolk of the unincubated egg. A study [16] has shown effects on progeny chicks from hens fed different levels of dietary P, affecting bone strength at hatch. While embryos can draw on Ca and some other minerals from the egg shell, the amount of P available is only that which was deposited in the egg yolk during oocyte development in the hen. Other minerals with importance for bone formation (e.g Cu, Zn) may be similarly affected [17].

Conclusions

The influence of the hatchery on subsequent broiler health and performance is a complex issue which continues to increase in importance as the lifespan of the commercial broiler becomes progressively shorter. There are significant differences between the incubation responses of the two major commercial broiler strains in use worldwide and this needs to be carefully considered when research results are published in this area. Departing from the accepted standard incubation temperature for the Cobb broiler by starting at a lower EST has been shown to improve leg strength without compromising hatchability. This approach is not advisable for the Ross strain, where the established standard program works well.

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O[41] Eggshell Temperature Patterns During Incubation on Tibia Characteristics of Broiler Chickens at Slaughter Age

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Abstract

Suboptimal leg health, occurring in a part of the fast growing broiler chickens, might be related to developmental imbalance between high growth rate and immaturity of leg bones. Improving leg bone quality may support the welfare and performance of broiler chickens. Eggshell temperature (EST) during incubation is one of the factors possibly affecting bone development and leg health in later life. This study was designed to evaluate effects of a combination of different EST patterns in week 2 and week 3 of incubation on tibia development of broiler chickens. A total of 468 Ross 308 eggs were incubated in one incubator at an EST of 37.8°C from embryonic day 0 (E0) to E7. Thereafter, eggs were incubated at a normal (37.8°C) or high (38.9°C) EST from E8 to E14, following a normal (37.8°C) or low (36.7°C) EST from E15 till hatch, resulting in a 2 x 2 factorial arrangement. After hatching, chickens were reared until slaughter age in a complete randomized block design with 4 EST groups and 8 replicates per group. At slaughter (d 41 or 42), two male chickens per replicate were randomly selected and tibia bones were obtained. Tibia weight (TW), length (TL), thickness (TT), osseous volume (TOV), pore volume (TPV), total volume (TTV), mineral content (TMC), mineral density (TMD), breaking strength (TBS), and stiffness (TSF) were determined. A high EST (38.9°C) in week 2, followed by a normal EST (37.8°C) in week 3 resulted in higher TW (Δ=+0.4-0.9 g; P=0.04), TMC (Δ=+0.2-1.7 g; P=0.001), TMD (Δ=+0.01-0.05 g/cm^2; P=0.001), TBS (Δ=+2.2-29 N; P=0.003), and TSF (Δ=+3.1-29 N; P=0.05) compared to the other 3 EST groups. A high EST (38.9°C) in week 2 resulted in a higher TT (Δ=0.06 cm; P=0.04), TOV (Δ=5.0 cm^3; P<0.001), TPV (Δ=0.7 cm^3; P=0.04), and TTV (Δ=5.8 cm3; P<0.001) than a normal EST (37.8°C). It can be concluded that 1.1°C higher EST than normal in week 2 of incubation stimulates tibia morphological, biophysical and mechanical characteristics at slaughter age. Looking at the week 3 of incubation, 1.1°C lower EST did not influence these characteristics.

Keywords: Eggshell temperature, incubation, tibia characteristics, broiler chickens

O42 Effect of High and Low Eggshell Temperature in Different Periods of Incubation on Hatch Result, Hatch Time and Chick Organ Development

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Abstract

This study was conducted to evaluate the effect of eggshell temperature (EST) in different periods of incubation on embryonic mortality, hatchability, hatch time and chick organ development. A total of 3150 hatching eggs were collected from Ross 308 commercial broiler breeder flocks at 32 wk of age. Hatching eggs were subjected to Low (L; 36.9°C), Control (C; 38.1°C), and High (H; 39.2°C) EST during 0-6, 7-12, or 13-18 d of incubation. In the study there were 7 groups, which were LCC, CLC, CCL, CCC, HCC, CHC, CCH according to the temperatures of the three periods (0-6, 7-12, and 13-18 d) of incubation. Hatching time was determined in each group by counting all chicks that hatched at 474 h (Early), 490 h (Middle), and 514 h (Late) of incubation. Fertile hatchability, embryonic mortality, and second quality chick percentage were calculated for per tray. Fifteen randomly selected chicks from each group were weighed and killed to determine residual yolk sac weight, heart, gizzard, liver, and bursa fabricus at 514 h of incubation. CCC group had the highest fertile hatchability, but the lowest one was HCC group ($P<0.05$). Other groups were ordinary from high to low; CCL, CCH, LCC, CLC, and CHC, respectively. Hatch time was affected from temperature of the first 6 d of incubation period more than other periods of incubation. Gizzard percentage was the highest in HCC group, but liver and bursa fabricius percentage were decreased due to High EST in different periods compared to other groups ($P<0.05$). Consequently, High EST during first 6 d of incubation had negative affect when compared with Low or Control EST groups. Therefore, it is important to calibrate machines before set the eggs, as well as to adjust the optimum level of the EST, the staff needs to check the EST in the first few days to achieve well hatch results and good chick quality from incubation process.

Key word: eggshell temperature, embryonic mortality, hatchability, hatch time, chick organ development

IS[18] Anti-Inflammatory Capacity of Phytogenic Compounds to Improve the Resilience of Broilers Against Intestinal Infections

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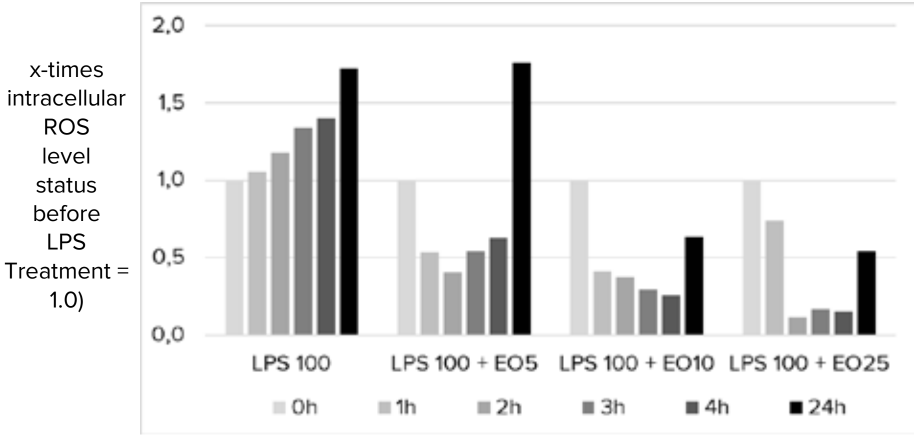
Abstract

Necrotic enteritis causes a strong reduction in feed intake and growth performance of broilers within 24-h after inoculation with a pathogenic *Clostridium perfringens* strain (1). This effect can be alleviated by adding sodium salicylate to drinking water of broilers (2), supporting the 'non-antibiotic, anti-inflammatory effect' concept of anti-microbial growth promoters (3). This was the basis for testing the anti-inflammatory effects of essential oils of the Myrtaceae plant family as an ingredient for a phytogenic feed additive to improve the resilience of broilers against intestinal infections.

The efficacy of the essential oil blend (dose levels 0, 5, 10 and 25 mg/L) was tested *in vitro* in a Caco-2 cells: On day 1 cells were plated overnight (150.000 cells/well) in growth medium in 96 well plates. On day 2, graded levels of the essential oil blend were added to the wells. On day 3, first the reactive oxygen species (ROS) detection reagent was added (Sigma Aldrich MAK143) followed by bacterial lipopolysaccharides (LPS) at t=0. Green fluorescence was measured with a multiwell plate reader at different time intervals during 24h. LPS induced a cellular inflammatory response within 90 min after incubation (Figure). Adding 5 mg/L of the essential oil blend reduced the intracellular reactive oxygen species for 90 min, whereas higher dose levels prolonged this effect.

Figure. The effect of graded levels of an essential oil blend from the Myrtaceae plant family on the development of the intracellular reactive oxygen species in Caco-2 cells during 24-h after LPS challenge (100 ng/mL).



Subsequently, this essential oil blend was included in a phytogenic feed additive that was tested in a necrotic enteritis infection model at Southern Poultry Research (Athens, USA). Male Cobb 500 broilers were housed in floor pens on built-up litter (7 replicate pens with 50 birds/pen). All birds were spray-vaccinated with Coccivac B (label recommended dose) on day 1 and infected with *Clostridium perfringens* on days 19, 20 and 21. A three-phase corn/soy diet was fed (starter crumble 0-21 days, grower pellet 22-35 days and finisher pellet 36-42 days). Production performance was measured, lesion scores and oocysts shedding (as oocysts per g (OPG) feces) at day 21. Treatments and results are given in Table.

Table. The effect of a phytogenic feed additive (PFA) on production performance, necrotic enteritis incidence and oocysts shedding in Cobb 500 broilers.

	1-21 days		1-42 days		Necrotic enteritis		OPG
	BW, g	FCR	BW, g	FCR	Mortality, %	Lesions	
NINT	603 [a]	1.430 [b]	2219 [a]	1.789 [b]	0	0	89000 [a]
INT	533 [b]	1.495 [a]	2056 [b]	1.866 [a]	14	1.1	80500 [a]
IT (BMD, 50 g/T)	559 [ab]	1.464 [b]	2138 [ab]	1.781 [b]	2.3	0.7	90600 [a]
IPFA (400 g/T)	574 [a]	1.436 [b]	2192 [a]	1.754 [b]	2.3	0.5	46400 [b]

NINT: Not infected with *Clostridium perfringens*, not treated; INT: infected with *Clostridium perfringens*, not treated; IT: infected with *Clostridium perfringens*, treated with bacitracin; IPFA: infected with *Clostridium perfringens*, treated with a PFA.

The phytogenic feed additive improved body weight gain, feed conversion ratio and reduced necrotic enteritis incidence to levels at least similar to the treated control (IT), whereas oocysts shedding was significantly reduced.

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O[43] Meat Quality, Digesta Viscosity, and Development of Footpad Dermatitis in Broiler Chickens Fed Gradually Increasing Dietary Phytogenic Feed Additive Supplemental Levels

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Abstract

The current study was conducted to evaluate the effect of graded dietary levels of a phytogenic feed additive (PFA) on growth performance, intestinal viscosity, and incidence and severity of footpad dermatitis (FPD) in broilers. Four hundred eighty, one-day-old male broilers (Ross 308) were assigned to four experimental groups, each consisting of 8 replicate pens (15 chicks per each replicate pen). Corn-soybean meal-based diet was formulated for starter, grower, and finisher phases, fed to the control group (without supplementation). Basal diets were supplemented with PFA at 100, 125, and 150 mg/kg levels, respectively. Growth performance parameters such as body weight gain, feed intake, and feed conversion ratio (FCR) were recorded. One bird was slaughtered from each pen at the end of experiment. Meat quality parameters including pH, color, drip loss, and cooking loss were measured on d 42 at slaughter, 24-h and 72-h post-slaughter, respectively. Intestinal viscosity and litter moisture were measured on day 42. All the birds were subjected to FPD scoring on d 21 and 42 of the experiment. Feed intake, BW gain, and FCR were not different among the dietary treatments throughout the study. Dietary PFA supplementation did not affect the intestinal viscosity, litter moisture, and FPD score in broilers. Although none of the addition levels were helpful in improving the growth performance, meat quality, intestinal viscosity, litter moisture, and in decreasing the FPD score, more research is needed to set the optimum dietary levels of blending these compounds for their prospective use in broiler nutrition.

Key words: Broilers; growth performance; footpad dermatitis; phytogenic feed additive

Introduction

Footpad dermatitis (FPD) is a contact dermatitis of broilers appearing at the plantar surface of foodpads of poultry. Many pre-disposing factors are associated with the development of FPD in poultry, main determinant being the litter moisture often arising due to the features related to poultry diets (1). Quite often, the poultry diets increase the intestinal viscosity of broilers, which in turn, increases the litter moisture that helps in the development of FPD in broilers. Energy (barley and wheat) and protein sources (soybean meal) contain non-starch polysaccharides (NSPs) (2, 3) that are indigestible in monogastric species. The soluble NSPs increase the intestinal viscosity of broilers, litter moisture, and enhance the development of FPD in broiler chickens (2). Phytogenic feed additives (PFAs), commonly used as plants, botanical extracts or essential oils, have been tested in different animal species like ruminants and non-ruminants especially in swine and poultry nutrition. The nutritional benefits of PFAs include the improvement in the performance of birds through better feed conversion ratio (FCR) (4), improved daily weight gain (5), better digestibility of fat, protein, calcium, and phosphorus (6, 7), and improved apparent total digestibility of crude protein (8). In addition, the dietary supplementation of PFAs may stimulate the blood circulation, digestive enzyme production, and reduce the load of pathogenic bacteria (9). Keeping in view these positive effects of PFAs on nutrient digestibility and potential antioxidative properties, it is hypothesized that PFAs can optimize the meat quality, gut viscosity, litter moisture, and can reduce the development of footpad dermatitis (FPD) in poultry. Moreover, to the best of our knowledge, there is no report available describing the effect of PFAs on gut viscosity, litter moisture, and the incidence and severity of FPD in broilers. Therefore, the present study was conducted to investigate the effect of gradually increasing the dietary PFAs supplemental levels on gut viscosity, litter moisture, and incidence and severity of FPD in broilers.

Materials and Methods

The present study was carried out at the Poultry Research Unit of Adnan Menderes University, Aydın, Turkey in conformity with the standards of animal welfare standards of animal care and use committee of the university. A total of 480 male Ross 308 one-d-old broiler chickens were purchased from a local commercial hatchery (Egetav Tavukçuluk San. ve Tic. A.Ş., İzmir, Turkey). The chicks were randomly placed in 32 pens of four experimental treatments consisting 8 replicate pens per treatment with 15 chicks per pen. The trial lasted for 42 days. Each pen had a 6 to 8 cm deep layer of wood shavings as a bedding material. *Ad libitum* access to feed and water was ensured throughout the experiment. Continuous lighting (24-h) was provided, no fluorescence light was used during the day time. The birds were maintained at 32ºC in the first week. A temperature of 0.5 ºC per day was reduced after the first week until a constant temperature of 24 ºC was achieved. The birds were visited four times a day and the mortality was recorded if any. Corn-soybean meal-based basal diet was formulated for broilers according to the recommendations by Aviagen for Ross 308 broilers during starter (d 0-10), grower (d 11-24), and finisher (d 25-42) phases (Table 1). The basal diet was fed to one of the four experimental groups allocated as control group. The other dietary treatments were obtained by supplementing the basal diet with phytogenic product at 100, 125, and 150 mg/kg, respectively. The phytogenic product used in this study was a unique blend of herbs, essential oils, and functional flavors in a powdery form characterized by menthol and anethole (Digestarom® Poultry; BIOMIN Holding GmbH, Getzersdorf, Austria). The product contained cinnamon 20 g/kg, cumin 20 g/kg, peppermint oil 170 g/kg, garlic oil 50 g/kg, anise oil 50 g/kg, fennel oil 40 g/kg, and SiO_2 and NaCl as carrier. Feed intake, BW gain, and FCR were reported for the periods 1-21 days, 22-42 days, and 1-42 days. The FCR was adjusted by adding the body weight of dead bird in the pen weight. The indicators of meat quality were measured at slaughtering on d 42, and 24-h and 72-h post-slaughter. In order to measure the meat quality parameters 24-h and 72-h post-slaughter, approximately 100 g of breast meat (pectoralis major) was collected after slaughter from each carcass in labelled plastic bags. The meat samples were stored at -18 ºC in a deep freezer (Bosch, Germany) until further analysis. Breast pH and colour measurements were recorded at slaughter and 24-h post-slaughter. Water holding capacity (WHC) of chicken meat depends on the storage time. Therefore, it was recorded 24-h and 72-h post-slaughter. WHC was measured in terms of two different values namely drip loss and cooking loss according to the procedure previously described by Honikel (10). The viscosity of intestinal contents (digesta) of broilers slaughtered on d 42 was measured. The samples of intestinal contents were collected from the anterior (duodenum and jejunum), and posterior (ileum) portions of the intestinal tract in separate micro-centrifuge tubes. Viscosity of each samples was measured using a Brookfield DV-E digital viscometer (Model Cone and Plate LVDV-E, Brookfield Engineering Laboratories Inc., Middleboro, MA.) according to the technique described by Bedford and Classen (12). Viscosity was measured in centipoise (cP). Approximately 100 g litter sample was collected from each pen in plastic bags at the end of the experiment. A known quantity of each sample was dried at 65 ºC for 24 hours. The samples were again weighed after drying. The incidence and severity of FPD was evaluated on days 21 and 42 of age by observing all the birds. A visual FPD scoring system was used described by Bilgili et al (13). Data were statistically analyzed using one-way analysis of variance (ANOVA) in a completely randomized design to evaluate the effect of different levels of PFA on various parameters of broilers. In a statistical software package (SPSS Version 17.0; SPSS Inc., Chicago, IL, USA). Means were compared using Tukey's post-hoc test. The confidence interval was set at 95% ($P < 0.05$). The results were presented as Mean $\pm$ SEM.

Table 1. Composition of basal diets for broiler's starter, grower, and finisher phases

Ingredients	**Starter**	**Grower**	**Finisher**
		%	
Corn	55.63	56.15	33.50
Soybean meal (48% CP)	37.50	36.00	33.50
Vegetable oil	2.50	4.15	4.25
Limestone	0.89	0.85	0.80
Dicalcium phosphate	2.30	2.00	1.73
Salt	0.35	0.35	0.35
DL-Methionine	0.37	0.25	0.12
L-Lysine sulphate	0.21	-	-
Vitamin-mineral premix[1]	0.25	0.25	0.25
Nutrient Composition of Diets (%)			
Crude protein	21.75	20.89	19.92
Metabolizable energy (Kcal/kg) (Calculated)	2910	3029	3070
Crude fiber	3.47	3.39	3.30
Crude ash	5.70	5.33	4.95
Calcium	0.97	0.88	0.79
Available phosphorus	0.54	0.48	0.43
Digestible lysine (Calculated)	1.17	1.02	0.96
Digestible methionine (Calculated)	0.66	0.54	0.40

[1]Vitamin and mineral premix (per kg of diet): retinol acetate, 1706 mg; cholecalciferol, 41 mg; DL-α-tocopherol, 27 mg; menadione, 0.99 mg; cobalamin, 0.015 mg; folic acid, 0.8 mg: D-pantothenic acid, 15 mg; riboflavin, 5.4 mg; niacin, 45 mg; thiamine, 2.7 mg; D-biotin, 0.07 mg; pyridoxine, 5.3 mg; manganese, 90 mg; zinc, 83 mg; iron, 121 mg; copper, 12 mg; iodine, 0.5 mg; selenium, 0.3 mg

Results and Discussion

The present study showed that BW gain, feed intake, and FCR of broilers were not statistically different among the treatments. These results are no different than those reported by Botsoglu et al (14) and (15) who found that dietary supplementation of oregano essential oil (100 and 50 mg/kg, respectively) did not affect the broilers' performance. Likewise, some other studies reported no effect of dietary PFAs on the performance of broilers (16, 17). In contrast, some studies have reported negative effects of dietary PFAs on the performance of broilers (8, 18). However, some other studies showed that dietary PFAs supplementation improved the performance of broilers. Jamroz et al (19) reported that the inclusion of 100 mg PFAs (containing carvacrol, cinnamaldehyde, and capsicum oleoresin) per kg of the diet improved the performance of broilers. Spernakova et al (20) reported similar findings in broilers fed rosemary powder at 500 mg/kg of the diet. Fortification of broiler diet with a blend of phytogenic extracts at 1 g/kg also increased the BW gain and enhanced the performance (21). The actual mechanism of action by which PFAs improve the performance of broilers is not known, however, it may be due to the stabilization of feed components, improvement of the gut environment, concentration of enviable microflora or by the continuous stimulation of pancreatic and the digestives enzymes (4). In addition, the composition of the diet as well as the genetic potential of the broilers also determine the efficacy of PFAs.

Table 2: Meat quality at slaughter and 24-h post-slaughter of meat of broilers fed different levels of PFAs (n=8)

Groups[1]	At Slaughter				24-h Post-slaughter					
	(L*)	(a*)	(b*)	pH	(L*)	(a*)	(b*)	pH	Drip loss (%)	Cooking loss (%)
Control	49.40±1.9	5.15±0.6	1.28±0.5	6.06±0.1	56.51±0.7	2.11±0.5	4.61±0.5	5.66±0.0	12.93±0.9	30.92±1.7
PFA100	51.41±0.4	5.72±0.5	1.80±0.4	6.20±0.1	58.60±1.0	1.70±0.4	4.50±0.5	5.61±0.0	12.31±0.7	27.82±1.9
PFA125	50.98±0.7	4.64±0.3	1.47±0.9	6.10±0.0	53.53±1.2	1.72±0.4	4.80±0.7	5.67±0.0	12.15±1.5	28.77±0.9
PFA150	50.41±0.4	3.84±0.3	2.56±1.4	6.02±0.1	56.80±2.1	1.84±0.4	5.56±0.8	5.65±0.0	13.10±1.0	29.30±0.9
P-value	0.585	0.164	0.826	0.662	0.114	0.918	0.700	0.465	0.915	0.521

[1]Control: 0 mg/kg PFA; PFA100: 100 mg/kg PFA; PFA125: 125 mg/kg PFA; PFA150: 150 mg/kg PFA

It is a general perception that the pre-slaughter stress conditions greatly deteriorate the meat quality such as color, pH, drip loss, and cooking loss. PFAs are believed to possess antioxidative properties that protect the cellular function in the intestine and other body tissues (22, 23). The antioxidative properties of PFAs may help the broilers overcome the negative effects of stressful conditions and later on during the storage time as well. Therefore, dietary PFAs may improve the meat quality of the chicken. However, in the present study, there was no significant effect of different levels dietary PFAs on the meat quality of broilers compared to the control (Table 2 and 3). These findings are consistent with those of Li et al (24) who reported that different levels of a commercial phytogenic product (0.5 and 1 g/kg in diet) had no effect on the pH, color, water holding capacity, and drip loss of meat of broilers in comparison with those fed control diet.

Table 3: Meat quality 72-h post-slaughter, intestinal viscosity, and litter moisture in broilers fed different levels of PFAs (n=8)

Groups[1]	72-h Post-slaughter						Intestinal viscosity (cP)		Litter Moisture (%)
	(L*)	(a*)	(b*)	pH	Drip loss (%)	Cooking loss (%)	Anterior	Posterior	
Control	55.75±0.9^{ab}	1.71+0.5	5.78±0.3	5.70±0.0	15.68±2.5	29.05±0.9	1.49±0.08	1.63±0.14	1.50±0.07
PFA100	57.79±1.0^{a}	1.53+0.3	6.05±0.9	5.61±0.0	12.87±0.7	28.89±0.9	1.47±0.13	1.48±0.09	1.48±0.07
PFA125	52.62±1.0^{b}	1.31+0.4	5.60±1.0	5.80±0.1	11.44±0.5	26.37±0.4	1.54±0.14	1.54±0.07	1.54±0.08
PFA150	54.94±1.1^{ab}	2.10+0.6	6.10±0.9	5.64±0.0	11.25±0.6	28.03±0.6	1.41±0.08	1.47±0.08	1.45±0.06
P-value	0.012	0.735	0.974	0.252	0.113	0.064	0.91	0.66	0.84

[1]Control: 0 mg/kg PFA; PFA100: 100 mg/kg PFA; PFA125: 125 mg/kg PFA; PFA150: 150 mg/kg PFA

Table 4: Incidence and severity of footpad dermatitis (FPD) score of broilers (at days 21 and 42) fed different levels of PFAs

Groups[1]	Day 21				Day 42			
	0	1	2	T[2]	0	1	2	T[2]
Control	20.54±4.46	46.72±1.78	32.75±4.28	79.46±4.46	32.96±5.56	47.21±4.43	19.83±6.00	67.04±5.56
PFA100	31.20±9.08	39.65±5.48	29.15±9.73	68.80±9.08	39.78±9.15	35.10±4.40	25.13±7.09	60.22±9.15
PFA125	23.82±6.98	44.78±4.75	28.77±4.76	73.55±6.76	39.19±7.23	44.46±4.37	16.35±7.68	60.81±7.23
PFA150	28.57±6.63	42.32±4.10	29.11±6.40	71.43±6.63	36.13±8.72	43.00±5.33	20.88±8.57	63.87±8.72
P-value	0.708	0.675	0.970	0.734	0.921	0.310	0.868	0.921

[1]Control: 0 mg/kg PFA; PFA100: 100 mg/kg PFA; PFA125: 125 mg/kg PFA; PFA150: 150 mg/kg PFA
[2]T = Total FPD lesion comprising of score 1 and 2

There is no report available describing the effect of PFAs on intestinal viscosity, litter moisture, and incidence and severity of FPD in poultry. Therefore, the results of the present study cannot be explained in the light of literature available. Intestinal viscosity of broilers depends on feed composition. Diets fed to the broilers, in the present study, consisted of corn and soybean. Soybean meal contains considerable amount of soluble and insoluble non-starch polysaccharides (NSPs) (25). An increase in the concentration of soluble NSPs generally increases the intestinal viscosity. This happens due to reduced nutrient digestion and absorption (26), consequently altering the gut physiology and intestinal ecosystem (27). Higher intestinal viscosity increases the litter moisture as the droppings become stickier. The droppings are caustic in nature, and prolonged adhesion of the droppings to the epidermis of paws elicits irritation. This results in the corrosion of epidermis and keratin layers of the paws causing a pathologic condition named FPD (28). PFAs are known to improve the nutrient digestibility (6, 7) that may, in turn, decrease the intestinal viscosity and litter moisture eventually. In the present study, it was expected that the PFAs supplementation at different levels in broiler diets would reduce the intestinal viscosity of the broilers resulting in lowered litter moisture, and FPD incidence and severity. However, it seems that these events did not occur in the present study (Table 3 and 4). The possible explanation is that the basal diets were similar in all the treatments so did the intestinal viscosity, litter moisture, and FPD score. In addition, the minimum concentration of active biomolecule to show its possible effect on intestinal viscosity, litter moisture, and incidence and severity of FPD is questionable.

Conclusions

The efficacy of PFAs is governed by a number of factors, and therefore, the use of PFAs in poultry diets should be paid attention only after careful considerations. The concentration of active biomolecules and the inclusion level is the key determining factor for growth performance and other goals of rearing the poultry birds. Hence, the inclusion level of PFAs in poultry diets may be different for different goals such as for maximal BW, maximal feed efficiency, and disease prevention etc.

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O[44] The Efficacy of an Engineered Biocarbon in Young Broilers During an Aflatoxin Exposure

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Abstract

This study was conducted to evaluate the efficacy of a carbonaceous biomass (Bio-C) on aflatoxins (AF) *in vivo* and *in vitro*. In *in vitro* adsorption test, a working solution was prepared at a concentration of 88 mg/kg (AFB1) in acetonitrile and then diluted with deionized water to the treatment concentrations of 0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg. A 0 mg blank solution was also prepared using deionized water as a control. To each of the 5 mL of AFB1 test solution tubes (with the concentrations 0.0, 0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg), 10 mg of the Bio-C was added (0.2%). The amount of absorbed AFB1 was measured with UV-visible spectrophotometry. For the feeding trial, a total of 192 Cobb-500 male broilers were obtained on the day of hatch and randomly allocated to one of 32 treatments pens (6 birds/pen). Birds were fed a broiler starter mash diet containing either 0 or 0.5 mg/kg AF, with or without 0.4% of Bio-C, resulting in 4 treatments arranged as a 2x2 full-factorial. Pen weights and feed consumed were recorded at 0, 7, 14, and 21 days of age, and mortality was recorded daily. On day 21, three birds from each pen were killed by cervical dislocation and the liver, kidney, and spleen were removed and weighed for relative organ weight assessment. Data were analyzed as a 2x2 full-factorial for AF level and Bio-C using the GLM procedures of SPSS, with a significance defined at $p \leq 0.05$. The results from *in vitro* adsorption test indicates that Bio-C adsorbed an average of 44% of AFB1 in solution of the range of doses. For the feeding trial, the results showed that the performance of birds receiving 0.5 mg/kg AF were not significantly different compared to the control group at any point during the 21 day trial. No main effects were seen on the performance parameters by the inclusion of Bio-C in the diet. There was also no AF/Bio-C interaction. The relative weights of the liver, kidney, and spleen were not significantly different whether birds were fed 0.5 mg/kg AF or 0.4% Bio-C. These data suggest that supplementation of Bio-C has no effect on broiler performance when provided in diets either free of AF or containing up to 0.5 mg/kg even though it has adsorption abilities *in vitro.*

Key words: aflatoxin, biocarbon, binder, broilers

IS[19] Meat Quality – White Striation and Woody Breast in Modern Broiler Production

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Summary

Recent reports show an increase in myopathies associated with fast growing broiler chickens all over the world. Rates (0 – 30%) appear to be flock dependent and related to factors such as nutrition, genetics, number of satellite cells at birth, and activity of the birds. The main three myopathies in broiler's breast fillets include white striation, woody breast, and spaghetti meat, which can appear individually or together. When meat is processed, these myopathies represent quality issues (firmer meat, lower water binding, aesthetics), but do not necessarily present a food safety issue. The industry is now working on reducing the incidences of these myopathies by learning more about genetics, nutrition as well as the interactions between environmental and management factors. Breeding programs are already showing some promising effects, and are expected to deliver more solutions in the future. There are also new advancements in automated sorting and detecting methods, at the meat plant, (e.g., near infrared scanners) to help processors. This presentation includes a systematic review of our current knowledge and some forecasting about future developments.

Keywords: chicken; genetics; meat; myopathy; nutrition; poultry

Introduction

Myopathies in fast growing broilers have been reported to increase all over the world. They mainly affect the breast fillet, but can also be seen in other areas (e.g., thigh). A report from 2016 estimated the cost of the white striation (WS) and woody breast (WB) myopathies at $200 million per year in North America alone (Kuttapan *et al*., 2016). More recent estimates suggest that this number is on the low side, especially when considering that, in the US, about 12.8 billion pounds of chicken breast meat are processed annually. At a retail cost of about $1.50 per pound, the market value of this product is $18 billion and $200 million only represents about 1% of this.

Overall, the industry has made big advancements in raising chickens faster and in a more efficient way; however, some suggest that this has also increased the incidences of some myopathies (Sihvo *et al*., 2017; Petracci *et al*., 2015; Barbut, 2015). White striation, for example, appears as parallel white stripes on the surface of the young broilers' *Pectoralis* muscle while the wooden breast syndrome results in accumulation of connective tissue fibers and fat cells in the inner muscle, causing firm texture of the tissue. Both myopathies have to do with damaged muscle cells replaced with collagen and fat tissue. According to literature, incidences of WS increase as body weight increases, especially above 2.7 kg. We also know today that it is related to the growth rate at a specific age period during growth of the bird, and not only to the absolute body weight. Different broiler's strains can also show differences in the frequency and severity of WS. Kuttappan *et al*. (2013) reported on four strains ranging from 5 to 35%. They also indicated that males showed higher number of severe WS compared to female birds (43 vs. 18%). However, it is important to note that newer data suggest when comparing males vs. females, body weight should also be taken into account since, at the same body weight, female birds tend to have a higher proportion of WS.

Overall, myopathies are a result of muscle cell damage that in the case of WB / WS do not have enough time for repair, especially in a fast growing bird. Usually satellite cells are taking part in the repair mechanism but it seems that they cannot keep up with the pace of the fast growing muscle. Another factor is growth rate and today growers know that decreasing it can minimize myopathies. Genetics also plays a role and currently breeding companies are working on selecting birds which are less susceptible to those myopathies. There is also a growing body of literature focusing on oxidative stress and insufficient oxygen supply to the fast growing pectoralis muscle that can enhance myopathy development.

Discussion

The WS and WB myopathies are the result of muscle fiber replacement by fat cells and connective tissue. In the past, it was more typical to see WS in older laying hens, but today it is seen in young broilers of 5-7 weeks of age. Fillets with WS also show myodegenerative muscle fibres, inflammatory cells and infiltration of eosinophilic material (Kuttappan *et al*., 2013); which points out to muscle cell injury and the beginning of repair. From a meat processing standpoint, WS fillets show higher cooking loss because they have less functional proteins. This is the result of some myofibrillar proteins, which have high water holding capacity, being replaced by connective tissue proteins (i.e., have low water holding). These fillets also show lower marinade uptake, when tumbled with brine, and result in overall lower cooking yield, because of the same reason.

Wooden breast muscles also showed an accumulation of adipose tissue cells and lymphocytes in a prevascular location (Sihvo *et al*., 2017). Results also indicate that WB starts focally and spreads to form a diffuse and more severe lesion. It was hypothesized that localized hypoxia is present in WB tissue due to vascular disruption and/or stagnant tissue perfusion (Bilgili, 2013). Sihvo *et al*. (2018) also reported that the transverse myofibre area, per vessel, was highest in the unaffected area of muscle from cases of focal WB. This was significantly higher than in macroscopically unaffected tissue, indicating that relatively decreased blood supply may trigger the development of WB in affected birds. As indicated before, the woody breast fillets are characterized by firmer texture of the harvested meat, and this can also be detected in the live bird. When meat is deboned one can observe the presence of a harder ridge when fillets are placed on a flat surface. Sometimes this is also accompanied with a paler appearance, some surface hemorrhages, and clear exudate on the surface. It should be mentioned that the severity of WS and of WB varies among birds within the same flock, and therefore simple scales have been developed to rank the severity. Sorting and / or grading are important to the industry, as fillets with a low degree of WS or WB do not pose a problem when processing/marketing the meat; however, if severity and proportion of WS/WB increases in the flock, some action is required. Today a number of processors are sorting the meat in their plants and diverting severe cases to specific operations.

Sanchez Brambila *et al*. (2016) reported that the sensory hardness of severe WS meat was significantly higher than normal and moderate WS meat. Tasoniero *et al.* (2016) noted that the presence of both WB and WS resulted in higher taste panel perception of toughness compared to normal meat, and instrumental toughness. However, when meat is ground and made into patties, no difference can be found. In any case, it should also be noted that some reports indicate that panelists can not detect differences after cooking of the meat.

Poultry companies are now looking at the effects of different factors on the occurrence of WS and WB. A large-scale study conducted by Bailey *et al*., (2015) reported a significant difference between two purebred lines of commercial broilers e.g., difference in selection history resulted in breast meat yield of 29 vs 21%. They showed that there were birds with a high genetic potential for increased body weight and below average for the WB problem. These birds are currently being used for selection for both traits in the desired direction. Overall, the authors reported that data analysis (from the two broiler lines: A and B) indicated that patterns of heritability and genetic correlations were similar. Heritabilities (h^2) of BW (body weight), PW (processing body weight) and BY (breast yield) ranged from 0.271–0.418; for DPM (deep pectoral myopathy) and WB h^2 <0.1; and for WS h^2 ≤0.338. Genetic correlations between BMM (breast muscle myopathies) and BW, PW, and BY were ≤0.132 in Line A and ≤0.248 in Line B. They also mentioned that the polygenic nature of these traits and the low genetic relationships with body weight and breast meat yield facilitates genetic improvement across these traits.

The proteome profiles of WB meat, in relation to meat quality traits, has been described by Cai *et al*. (2017). They reported that WB meat had significantly higher cooking loss than unaffected meat, more fat, moisture, less protein, as well as salt soluble proteins. Whole muscle proteome analysis indicated 8 proteins that were differently expressed between WB and unaffected fillets. It was suggested that the differences indicate an increase in oxidative stress in WB meat. Abasht *et al*. (2016) also looked at oxidative stress and focused on characterizing metabolic features of WB. The affected muscles showed increased oxidative stress, elevated protein levels, altered glucose utilization, and 140 muscle degradation biochemicals that were significantly different between WB and unaffected chickens. WB tissues showed a unique metabolic signature, which the authors suggested be used to identify candidate biomarkers for diagnostic purposes.

The impact of incubation temperature and time of hatch on broiler muscle growth and morphology was investigated by Clark *et al*. (2017) looking at adult myogenic satellite cells that initially develop in late-term embryos, and are the myogenic cells that repair damaged myofibres and increase post-hatch growth. They exposed eggs to 39.5°C for 0, 3, or 12 hr per day and showed a reduction in the number of broilers with moderate to severe myopathic attributes compared to control. Similarly, there were fewer late hatch birds with fibrotic and necrotic *P. major* muscles compared to the early hatch group.

Currently a few companies are developing equipment to evaluate and detect WB meat for high volume processing plants. This should help the industry dealing with line speeds of up to 15,000 broilers per hour (Barbut, 2015), and where a significant number of the broilers are deboned. Many plants are using manual inspection (employees inspecting and touching the deboned fillets). Putting employees on the line adds cost to the operation, therefore, finding a good semi/ fully automated inspection system is important.

Near infrared has been reported successful in sorting out WB meat (Wold *et al*., 2017). The detection is based on the fact that WB meat has lower protein and higher moisture content. The equipment, originally developed to determine feed composition, has been adapted to determine incidences of WB. The authors indicated that such an on-line system can be used to automatically sort chicken fillets, at a rate of a few hundred per min, to different product categories. The equipment is currently being used in a few large plants and fine-tuning is still going on. There is another company working on a similar concept, but they also reported incorporating calcium content which is also known to be different in WB samples.

Image analysis has been recently reported as another potential way to identify WB problems on the line; a patent was filed last March in the US. The idea in this case is that fillets affected by WB have a unique ridge which can be detected by a non-contact method.

At the moment, the industry is examining different approaches to minimize these myopathies. They range from working on nutrition to genetics, environmental factors and their interactions. Bodle *et al*. (2018) evaluated different dietary alterations to mitigate the WS and WB in high-yielding commercial male broilers. Their test diets included a commercial reference diet compared to several other diets (e.g., increasing the ratio of digestible arginine to digestible lysine; doubling the vitamin content; restricting amino acids). Overall, there was no difference in performance at the end of the starter phase; however, at the end of the grower and finisher phases, feeding lower amino acids grower diets suppressed body weight and increased feed conversion. These changes resulted in a shift in WB scores, showing the potential use of diet modification to reduce some of the WB myopathy. Abasht *et al*. (2016) also recommended vitamin C supplementation to enhance antioxidative metabolism and decrease the rate of WB. One of the challenges with the concept of reducing growth rate is the need to find alternative ways to supply the growing meat demand. A study released by the National Chicken Council (2017) mentioned that if only one-third of USA broiler chicken producers switched to a slower growing breed, nearly 1.5 billion more birds would be needed annually to supply the amount of meat currently produced. This will also require more feed to fill 670,000 additional tractor trailers on the road every year. The additional land needed to grow the corn and soybeans would be 7.6 million acres/year, or roughly the size of the state of Maryland.

Overall, processors who sort out the WS and WB meat divert it to products such as chunked and formed chicken nuggets, patties, and emulsion type sausages. In such products, the meat is ground and then hardness is not an issue. However, an interesting observation is that hardness of the WB fillets is decreasing over time and cumulative drip loss shows a different pattern from normal meat (Sun *et al.*, 2018).

In 2018 the Food Safety and Inspection Service reissued its disposition instructions for the poultry meat quality issues known as Wooden Breast and White Striation. The notice includes a detailed list of background information, responsibilities for how public health veterinarians, inspectors-in-charge, front line supervisors, and supervisory consumer safety inspectors ought to work with inspection program personnel "to identify and verify that poultry processors are removing trimmable inflammatory tissues that may be associated with these conditions...signs of inflammation associated with WB and WS include: swollen breast tissues; scattered, small blood spots or patches on the surface of muscles, particularly near the top or shoulder end of the breast fillet; and thick, often blood-stained fluid and signs of muscle deterioration". The notice also mentions that "inflammatory tissues must be trimmed owing to their being unfit for human consumption, whereas tissues not exhibiting active inflammatory signs, i.e., WS only, are considered safe and do not necessarily need to be removed". As indicated before, the presence of WS and WB by themselves have not been associated with a disease condition that poses health concerns (Bodle *et al*., 2018). It will be interesting to see how this issue will be dealt with later in the future.

In summary, there are various factors affecting the rate of WB and WS in commercial broiler flocks. In the short term, growers are currently engaged in activities such as diet modifications, management, and to some extent genetics. Long term solutions focus more on selecting bird lines which show less of the problem, and learning more about the interactions between environmental factors and these myopathies.

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O[45] Effects of Light Intensity and Experimentally Induced Deep Pectoral Myopathy Via Encouraged Wing Flapping on Lipid Peroxidation and Antioxidant Parameters in Broilers

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Abstract

Effects of experimentally induced deep pectoral myopathy (DPM) via encouraged wing flapping and light intensity on blood lipid peroxidation and antioxidant parameters were investigated in broilers. Half of the broilers were exposed to 5-lux light intensity one-week before slaughter age while the rest of the birds were kept at 20-lux. Broilers in each light intensity group were randomly divided into two groups and half of them were encouraged to wing flapping to induce DPM, experimentally. Therefore, the experimental arrangement consisted of 4 groups; 5lux DPM+, 5lux DPM-, 20lux DPM+, 20lux DPM-. The same procedure was repeated at 35, 39 and 43 d. Creatine kinase activity increased by slaughter age. Providing 5 lux of light intensity resulted in lower creatine kinase activity. There was no effect of treatments on MDA activity. There was an age-related decrease in SOD activity. The highest SOD activity was obtained for broilers slaughter at 35 d and exposed to 5-lux before slaughter. Effect of light intensity on SOD level was not significant when broilers were forced to wing flapping. DPM induction increased GSH-Px activity at 35 d compared to DPM- while DPM induction had no effect on GSH-Px at 39 and 43 d. The results showed that lower slaughter weight and 5-lux light intensity might reduce oxidative stress and muscle injuries.

O46 Investigation of the Effects of Dietary DL-Methionine and L-Carnitine Supplementation on Preventing White Striping in Broiler Breast Meat

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The study was aimed to investigate the effects of dietary supplementation of DL-methionine and L-carnitine on growth performance, breast meat weight/yield and occurrence of white striping in broiler breast meat. Effect of white striping on proximate composition of breast meat was also determined. A total of 480 one-d-old Ross 308 male broiler chicks were randomly divided into 4 experimental groups, each consisting of 8 replicates having 15 chicks in each pen. The study followed a design with 2×2 factorial arrangement of two levels of methionine (requirement level and %25 higher than requirement level) and L-carnitine (0 and 100 mg/kg). Corn/soybean meal-based basal diets were formulated for starter, grower, finisher 1, and finisher 2 phases of broilers in accordance with the recommendations of Ross 308 Manual (2014) to meet or exceed the nutrient requirements of male broilers. Dietary 100 mg/kg L-carnitin improved ($P<0,05$) feed conversion ratio 0 to 49 days. Other performance parameters were not different among the treatments. DL-methionine supplementation increased ($P<0,05$) the breast meat weight and yield in broilers at day 49. Fillets were visually scored for three degrees (normal, moderate, and severe) of white striping at 39 and 49 days of experiment. None of the dietary treatments had any significant effect on the incidence of white striping. Moderate white stripped breast meats exhibited higher fat content ($P<0,01$) and lower protein content ($P<0,001$), in comparison with normal breast meats. Moisture and ash levels in breast meat did not differ significantly with respect to white stripe severity.

Key words: Broiler, white striping, DL-methionine, L-carnitine

O[47] Strategies to Prevent on Pectoral Myopathies in Broiler Meat Production: Effects of Genotype and Floor Type

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Abstract

Pectoral myopathies such as white striping, woody breast, pale-soft-exudative meat, spaghetti meat and deep pectoral muscle myopathies are a major problem affecting the broiler meat industry. Pectoral miyopathies in broiler breast fillets have been increasing, which has generated concerns in the broiler meat industry regarding negative effects on consumer acceptance. The exact etiology is still unknown, but it is thought that myopathies can be reduced with different approaches specific to each. This study was conducted to investigate the effects of genotype and floor type on some pectoral myopathies such as whitestriping in broiler meat production and to develop strategies to prevent.

Poster Presentations

P[01] Interaction between Feed and Microbiota in Intestinal Health in Poultry of Turkey

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Abstract

It is an indisputable fact that animal protein sources are important in meeting the nutritional demands of the growing world population. Therefore, the importance of intestinal health is increasing day by day in order to make a healthy, efficient, high quality and cheap animal production. The importance of intestinal health is due to the fact that it provides maximum utilization of nutrients. The highest possible use of nutrients can only be achieved by a healthy digestive tract. Although the digestive system has the largest surface area in the organism, it is a complex system with useful useless, viable life-saving mechanisms to incorporate or defend against a large number of components. Therefore, any factor that may affect the health of the intestine has an impact on the overall health status, performance and quality of the resulting animal product. In general terms, as a result of the studies carried out in this concept, “intestinal health” is affected mainly by 3 factors. These factors are mainly; feed, digestive tract mucosa and microorganisms found in the digestive tract (microflora-microbiota) was revealed. However, we can say that diseases, environmental conditions and feed additives are effective in intestinal health. Intestinal health is a current and future workplace that can be addressed by a multidisciplinary association such as nutrition, microbiology, immunology and physiology. Especially in intestinal health, the relationship between feed-feed additives and intestinal microbiota is very important

P02 Alternative Feed Additives That can be Used Together with Antibiotics against Histomoniasis Disease in Turkeys

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Abstract

This review is based on the fact that alternative feed additives that can be used together with antibiotics against Histomoniasis disease in turkeys can be effective in the treatment process. With the prohibition of nitroheterocyclic compounds such as nitroimidazole, nitrazine, enheptin, the researchers lean to medicinal and aromatic compounds. It is of great importance that both the immune system is strengthened and alternative feed additives are used to prevent the cecum and liver lesions. Since the researches have revealed that in the illness of Histomoniasis, the deaths can result from liver dysfunction or peritonitis, in recent years the importance of liver protectors is increasing.

The photographs used in the review were taken by the author in the company that has 2000 turkey (Serik, Antalya).

Key words: Histomoniasis, bentonit, mannanoligosakkaritler, timol, karvakrol, silimarin

P03 Probiotics in the Control of Salmonella

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Abstract

By rearing of poultry, pathogens carried with chicks, feed, water and rodents grow in the habitats intestine and bedding. Salmonellosis is one of the most important foodborne illnesses. Although *Salmonella* serovars that cause human disease are commonly found on all kinds of food products, poultry is still considered as the major source. With effective monitoring and control programs, prevalence of *Salmonella* can be reduced. In this study, the effects of probiotic bacteria as spray on bedding and feed additive were investigated on *Salmonella* control. In *Salmonella*-positive broiler and turkey houses *Bacillus subtilis* (sprayed on bedding) and *Pediococcus acidilactici* (drinking water feed additive) suppressed *Salmonella* so that drag swab samples were *Salmonella*-negative. With probiotic use in poultry breeding, Salmonella can be controlled, food safety can be ensured, a high quality and economical production can be achieved. However, when the *Salmonella* source is not removed, *Salmonella* is found again when the probiotic application is terminated.

Key Words: *Salmonella*, probiotic bacteria, *Bacillus subtilis*

P04 Postbiotics

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Abstract

The use of probiotics as an alternative to antibiotics in animal nutrition is an important issue in the last decade. Probiotics are defined as live microorganisms that can provide a health benefit for the host when administered in appropriate and regular amounts. However, the efficacy of commercial probiotics and their strains is not always sufficient. The other concern about the usage of probiotics is they can transfer the antibiotic resistance gene to other organisms. Therefore, postbiotics, defined as probiotic metabolites, become an important subject that has been studied on. In this review, the postbiotic concept and the information about a number of studies about the effects of human and animal health are summarized.

Keywords: Antibiotic resistance, feed additives, postbiotic.

P05 Use of Some Natural Antioxidants in Poultry Meat

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Abstract

Antioxidants are used to minimize oxidative changes in poultry meat. Oxidative changes have some negative effects on the quality of poultry meat and also cause changes in their sensory and nutritive properties. The antioxidants used in the food industry are classified as natural and synthetic antioxidants. Natural antioxidants are often preferred because of the toxicological and carcinogenic effects of synthetic antioxidants and because of consumers demands for natural food additives. Due to the high phenolic compound content, some fruits and plant parts are used as natural antioxidants. In this study, we will be given information about oxidative deterioration in poultry meat and fruit and plant extracts used as natural antioxidant against this degradation.

Keywords: Antioxidant, Lipid oxidation, Natural antioxidants, Poultry meats

P[06] Typing of Rare Isolated *Salmonella* Serotypes with MLST

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Abstract

The most common serotypes are *Salmonella enterica* subsp. *enterica* serotype Enteritidis and Typhimurium in *Salmonella* infections which is one of the major problems of poultry producers. In recent years, the prevalence of these serotypes have decreased with the control programs applied in Turkey as many countries to control salmonellosis cases, the isolation rates of other serotypes have increased especially *Salmonella* Infantis. This suggests that rare *Salmonella* serotypes may become dominant in the next years. It is necessary to investigate the epidemiology of these serotypes and the importance of molecular typing methods has increased that will provide data to these studies. In this study, it was aimed to evaluate the *Salmonella* serotypes which are rarely seen in our country as in the whole world by Multilocus Sequence Typing (MLST). Sixteen strains of *Salmonella* were used that isolated from broiler chicken dust samples between 2015-2018 as *Salmonella* Bazenheid, *Salmonella* Burgas, *Salmonella* Gombe, *Salmonella* Hayindogo, *Salmonella* Jamaica, *Salmonella* Kamoru, *Salmonella* Paris, *Salmonella* Stuttgart. MLST analysis was performed on 7 housekeeping genes to each strain. In this study for the first time in the world, *S.* Bazenheid, *S.* Burgas, *S.* Gombe, *S.* Hayindogo, *S.* Jamaica, *S.* Kamoru, *S.* Paris, *S.* Stuttgart serotypes were typed by MLST and sequence types were determined.

Key Words: Poultry, Rare *Salmonella* serotypes, *Salmonella* Multilocus Sequence Typing.

P07 Should We Change Our Poultry Meat Production Process? An Article About Productivity

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Abstract

As of 2016 in Turkey, 1.958 million tons of chicken meat is produced. Total poultry for chicken meat; 11 - 11.5 million m^2 is calculated. In the current production model, only one third of the total area is used in the first 10 days of chicken production. Each set in the period of 1000 m^2 per 40 - 45 tons of live production is made. There are 5.5 and maximum 6 periods in 365 days. 240 - 270 tons of live production per year is possible. Each 1000 m^2 poultry house; It contributes to the production of chicken meat around 170-190 tons per year.

In this article, can we grow by 20% without having one square meter of house construction in our country? We're opening the question for discussion.

P08 Changes in Lipid Fraction of Chicken Doner During Cooking

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Abstract

In this study, the change in lipid fraction due to the heat treatment applied during the production of chicken doner was investigated. For this purpose, the samples were taken from four different chicken doner kebab block before and after cooking. Moisture content, oil content and free fatty acid content of raw and cooked doner samples were determined according to AOAC 950.46 method, Soxhlet extraction method and AOCS (2000) titrimetric method (Ca 5a-40), respectively. Lipid fraction was extracted from the raw and cooked samples using Folch extraction method. Conjugated diene, specific absorption (K_{232} and K_{270}), peroxide and TBA values were analyzed for lipid oxidation products.

According to the results, the maximum center and surface temperatures of chicken doner during cooking were found as 10.6 °C and 80.3 °C, respectively. L*, a* and b* values of chicken doner samples were determined as 75.21, 4.19 and 23.04 by Minolta colorimeter, respectively. The average moisture content of the raw doner sample decreased from 78.71% to 56.07% by cooking.

While the oil content of the raw doner was 10.92%, the oil content varied between 14.81-33.11% in cooked doner samples. It was found that the oil content of the samples increased as the cooking time increased towards the inner parts of the doner block. This increase is due to the chicken fat used during spitting of the doner meat on a stick. The difference between oil contents of the doner blocks depend on the raw material and the amount of oil dripping away during cooking.

While the amount of free fatty acid content of raw doner was 1.79%, it increased to 2.06% at the 3rd hour and it decreased to 1.33% at the 6th hour. Peroxide values of the doner samples were 3.2 meq O_2/kg oil in raw samples and it was found as 2.2-3.0 meq O_2/kg oil in cooked samples. On the other hand, the conjugated diene value of raw doner was 0.24% and it ranged between 0.14 and 0.30% in cooked samples. TBA value, which is an indicator of aldehydes, was found as 0.74 mg MA/kg in raw samples and 1.46-2.24 mg MA/kg in cooked doner samples. Oxidation products formed at low levels due to the low center and surface temperatures of the doner during cooking.

Lipid oxidation levels of chicken doners highly consumed in our country and in the world were examined and their health risks were evaluated in this study.

Key Words: Chicken doner, oxidation products, oil and moisture content

Acknowledgement: This study was supported by the Scientific Research Projects of Abant Izzet Baysal University with the project number of 2018.09.04.1273. We also would like to thank Erpiliç Integrated Poultry Production Marketing and Trade Limited Company.

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P09 Devolopment of Molecular Diagnostic Kit for The Rapid Diagnosis of *Salmonella* Enteritidis and *Salmonella* Typhimurium Serotypes

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Abstract

Salmonella enterica serotypes are identified according to the Kauffman-White scheme based on the somatic O antigen and flagellar H antigen which are present in the cell wall of *Salmonella*. The aim of this study is to develop a multiplex quantitative real time polymerase chain reaction (multiplex-qPCR) based molecular diagnostic kit for the rapid diagnosis of *Salmonella* Enteritidis and *Salmonella* Typhimurium serotypes, which are frequently isolated in the world. In this study, a total of 175 (30 *S.* Enteritidis, 35 *S.* Typhimurium, 55 *S.* Infantis, 22 *S.* Mbandaka, 12 *S.* Liverpool, 4 *S.* Newport, 17 *S.* Virchow strains) were used to test the specificity and sensitivity of the developed kit while *Escherichia coli* ATCC (25922), *Pasteurella multocida*, *Avibacterium paragallinarum*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus epidermidis, Listeria monocytogenes, S.* Mbandka, *S.* Hadar, *S.* II, *S.* Kentucky and *S.* Havana strains were used to optimize the method. The multiplex-PCR results were 100% compatible with the conventional serotyping results, and the specificity and sensitive ratio was 100%. It is assumed that the newly developed multiplex qPCR technique could be used as an alternative method to conventional serotyping in laboratories which do not have adequate infrastructure.

P[10] Usage and Functional Properties of Natural Ingredients in Poultry Meat Products

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Abstract

Poultry meat (chicken, turkey, goose, duck, quail, ostrich and similar poultry animals) have protein, fat, essential amino acids, minerals, vitamins and other nutrients and they have great importance in human nutrition because it contains a significant amount. The importance of consumption of red meat and cancer and the importance of poultry meat consumption have increased considerably. Fat reduction, salt reduction, the use of natural antioxidants and natural antimicrobials are important issues in recent years in order to provide functional properties to meat products. The aim of this study is to summarize the studies on this subject.

P11 Prevention of PSE Meat Problem in Broiler Breasts Meats with Using Ascorbic Acid

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Abstract

Pale, soft and exudative (PSE) chicken meats are growing problems for the poultry meat industry. Pale, soft and exudative (PSE) breast meat is result from a rapid postmortem pH decline (glycolysis) whereas carcass temperatures are still high. This decrease in pH values leads to protein denaturation and this case caused pale color and reduces water-holding capacity in chicken breast meat. Therefore, it was aimed to prevent the PSE meat problems adding 100 mg of ascorbic acids per kg to the chicken feed as an antioxidant source which might be reduced oxidative stress in muscles prior to slaughtering in our study. Moreover, three different groups (normal: 1, PSE: 2 and adding 100 mg ascorbic acids per kg: 3) were constituted. Changes in physicochemical parameters (color, pH and water holding capacity) of PSE meats were investigated throughout storage for 96 h at 2°C. The L* values of all samples were statistically difference both on all storage times and throughout storage times ($p<0.05$). The a* values of all samples were no significant differences on all storage times. The b* values of all samples were increased during storage periods. As expected, the PSE meats had lower pH value than normal meats. Throughout storage, it is determined that adding 100 mg of ascorbic acids per kg to the chicken feed helped to maintain of water holding capacity.

Keywords: PSE meat, chicken breast meat, ascorbic acids

P[12] Effects of Methionine Supplementation on Growth Performance and Meat Quality of Broilers

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Abstract

Methionine, along with lysine is the first limiting amino acid used in poultry rations. In this regard, the addition of methionine sources to broiler rations is often necessary. Methionine and other sulfuric amino acids are extremely important for metabolic functions such the synthesis of other sulfuric acids and the protection of cells against oxidative stress. The insufficiency methionine source in the ration may have negative effects on growth performance, feed conversion rate, breast meat yield and meat quality parameters. However, the use of different methionine forms (DL-methionine, DL-2-hydroxy-4-(metilthio) butanoic acid) at higher levels than necessary has no effect on growth performance, meat yield and quality.

Keywords: Broiler Chickens, Meat Quality, Methionine, Performance

P13 Economic Sustainability and Animal Welfare in Broiler Production

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Abstract

Sustainability is a multi-dimensional concept with economic, environmental and social aspects. Usage of sustainable production methods is essential for the maintaining continuity of broiler production. The sustainability of broiler production is linked to animal performance and welfare, and environmental burdens of the production. Sustainable broiler production must be economically viable; that means while the consumers can able to access affordable and good quality meat, the producers and other stakeholders of the chain can continue their investments. The present study aims to compare broiler production systems (conventional using fast-growing (G-H), slow-growing (B-Y) and fast-growing chicks (B-H) using plant-based feeds) based on their contribution to sustainable development of broiler production. Age at slaughter, final weight at slaughtering, feed consumption, feed conversion ratio, bedding cost, and net income were evaluated as economic criteria. Mortality rate, indoor density, litter moisture and pH and incidence of foot pad lesions were used as welfare criteria to compare the systems. In the light of the criteria evaluated, G-H production was found to be more sustainable compared to B-Y and B-H.

P[14] Evoluation of Chicken By-Products in Bioactive Peptides with Positive Effects on Health

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Abstract

Thanks to its high-quality protein content, tons of chicken are made ready for human consumption every year. In this process, by-products with high protein content are revealed. From the perspective of economic benefits, by-product management and sustainability, hydrolysates having bioactive properties from protein-rich by-products make it possible to design new functional products with high added value. Bioactive peptides can be obtained from the by-products in various ways and the most commonly used route is enzymatic hydrolysis. There are several studies about obtaining the bioactive peptides via various enzyme and enzyme combinations. Chicken-based bioactive peptides have antioxidant activity in addition to regulating physiological processes such as controlling blood pressure and blood lipid levels. In this situation, by-products such as chicken skin and chicken legs can be suitable raw material for the production of functional foods and nutraceutical substances.

Keywords: Chicken by-product, bioactive peptides, protein hydrolyzate

P15 Natural Alternatives for Avian Coccidiosis Control

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Abstract

Numerous studies have been conducted so far in the control of coccidiosis caused by Eimeria parasite. The most common method of protection is the use of anticoccidial chemical compounds in feeds. However, in the prevention of the disease, only the participation of antioxidial substances in the diet and the related expenditures alone are not sufficient but increase the cost. Furthermore, eimeria species have developed resistance to these chemicals and limit their long-term effects in the prevention of disease. All of this suggests that we need to develop new strategies for exploring alternative product effectiveness.

Keywords: Anticoccidial, Feed additive, Poultry feed

P16 Development Kit for Rapid Diagnosis of Common Isolated *Salmonella* Serotypes in Turkey

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Abstract

Salmonella species are identified at serotype level with surface Vi, somatic O and flagellar H antigens according to Kauffmann-White scheme. Conventional serotyping is an expensive, time-consuming method that must be performed by specialized personnel. Therefore, molecular-based serotyping studies have increased in recent years as an alternative. In this study, develop a quantitative real-time polymerase chain reaction (qPCR) based molecular diagnostic kit has aimed for rapid diagnosis of *Salmonella* Infantis, *Salmonella* Kentucky, *Salmonella* Hadar, *Salmonella* Mbandaka and *Salmonella* Senftenberg serotypes, which are frequently isolated in the world. A total of 205 strains were used to test the specificity and sensitivity of the developed kit as 17 *S*. Agona, 35 *S*. Enteritidis, 15 *S*. Hadar, 35 *S*. Infantis, 30 *S*. Kentucky, 12 *S*. Liverpool, 20 *S*. Mbandaka, 10 *S*. Senftenberg and 31 *S*. Typhimurium strains. For the optimization of the method, *Avibacterium paragallinarum*, *Burkholderia cenocepacia, Escherichia coli* ATCC (25922), *Pasteurella multocida*, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus pneumoniae* strains were used. Real-time PCR results were 100% compatible with conventional serotyping results. The specificity and sensitivity were determined as 100%. It is assumed that the newly developed quantitative real-time PCR technique could be used as an alternative method to conventional serotyping in laboratories which do not have adequate infrastructure.
Key Words: qPCR, real-time PCR assay*, salmonella,* serotype.

P17 The Presence and Importance of Coagulase Negative Staphylococci in Poultry

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Abstract

Staphylococcal infections are considered as one of the causes of infections in birds as well as in humans and animals. Staphylococcal infections, especially in the poultry sector, cause a decrease in weight gain and egg production, and an increase in mortality rates and lameness. Although Coagulase Negative Staphylococci (CNS) has long been considered as commensal and contaminant bacteria, recent studies have shown that some CNS species can produce enterotoxin and also have pathogenic effects in humans. The use of antibiotics in animal production has been associated with the development and dissemination of antibiotic resistant organisms, including commensal microorganisms. The non-pathogenic CNS species have been associated with opportunistic infections and antibiotic resistance. Coagulase negative staphylococci containing many species such as *S. hyicus, S. gallinarum, S. arlettae, S. chromogenes* and *S. xylosus* are frequently isolated from nostrils and skin of chickens. While CNS are considered as harmless microorganisms in chickens, it has been reported that CNS produced under suitable conditions have been transformed from apatogen to pathogen. *Staphylococcus hyicus*, *S. sciuri*, *S. simulans* and *S. epidermidis* are isolated from dermatitis and tendosynovitis cases. These species are seen as opportunistic in CNS infections. Although coagulase negative staphylococci constitute an important problem in terms of public health, in recent studies it has been determined that CNS isolated from poultry products had antibiotic resistance.

P18 Effects of Using Whey and Whey Powder in Broiler Feeds on Performance

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Abstract

As a byproduct of cheese processing whey has high nutritive value and functional properties. Various powder products are obtained from whey such as whey powder. Whey protein has high biological value. It is rich in lysine and leusine. Whey and whey powder has positive effects on broiler performance and intestinal microbial flora. There are no enough and detailed studies on this area. Detailed studies must be made to determine the effects of whey or whey powder supplementation on performance, immunite, bone quality characteristics, antioxidant activity, meat lipid oxidation parameters in broilers. Whey evaluation in various areas without wasting is also important in terms of the environment.

Keywords: Whey, Whey powder, Broiler, Protein source

P19 Effect of Coating Chicken Breasts Meat with Different Antimicrobial Agents on Shelf Life and *Salmonella* Count

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Abstract

In this study, the effect of coating of chicken breasts with edible coating (gelatin) with different antimicrobial agents and the effect of this coating on the shelf life of *Salmonella* and chicken breast meat was investigated. Antimicrobial oils and edible beef gelatin were used in the study. Meats were supplied from retail outlets. Approximately 5 log *Salmonella* were used for analysis without contaminating or contaminating. According to the natural antimicrobials used, groups were formed and plating was applied. Covering meats were covered with stretch film on styrofoam plates and kept at + 4 ° C in refrigerator conditions. Microbiological analyzes were performed on specific days (until day 15).

In the study, significant decreases were found in *Salmonella* in the groups using natural antimicrobials compared to the control groups ($p > 0.05$). It was determined that the shelf-life analysis was longer to the control group. According to the results of this study, it is recommended that natural antimicrobial coatings help to increase the shelf life of meats and therefore can be used in poultry processing plants.

P[20] Investigation of a Probiotic as an Alternative for Salinomycin Against Coccidiosis in Broilers

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Abstract

A probiotic containing *Pediococcus acidilactici* and *Bacillus subtilis* (Smart ProLive) at a 1x107 CFU/ml dose in drinking water were given continuously from the d 14 to the end of the treatment (d 35) in broiler chickens. Experimental infection was produced by oral gavage of sporulated *E. tenella* oocysts at 14th d of age. Probiotics appeared to be superior to salinomycin on the villus height and crypt depth of cecum and ileum ($p<0.05$). A numerical, but not significant ($p>0.05$) improvement on the LBW was determined at the groups of probiotic and salinomycin+probiotic than that of control and salinomycin groups. Nevertheless, FC and FCR results of the probiotic and salinomycin+probiotic groups were good than that of control and salinomycin groups. Probiotics were effective on the villus heights and crypt depths than that of salinomycin alone. Salinomycin appeared to be good only than control group in all the parameters. Not a significant difference from antibody titers was existed among the groups. Based on these results it can be concluded that a good source of probiotics can be used as natural antimicrobial growth promoters in replacement with forbidden anticoccidials in broiler rearing.

P21 Effects of Dietary Fiber on Intestinal Health of Broilers

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Abstract

Raw fiber is divided into two groups as water soluble and insoluble raw fiber according to physicochemical properties. Water soluble fibrous substances are beta-glucan, arabinoxylan, pectin, galactomannon; while water insoluble ones; major cellulose, hemicellulose, pectic substances, cell wall nitrogen and lignin. Water-soluble fiber fractions adversely affect bowel health and lead to serious reductions in animal performance. As a result of malnutrition of poultries, in terms of crude fiber, cannabismism and mortality rate increase. While making the formulations, taking into account the level and nature of the fibers contained in the ration is important for an economical poultry husbandry.
Keywords: Raw fibers, poultry, intestinal health, ration

P22 Effects of Different Monochromatic Lighting Applications on Growth and Some Behavior Characteristics in Japanese Quail

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Abstract

The aim of this study was to determine the effects of white (control group), green (560 nm), blue (480 nm) and red (660 nm) monochromatic lighting on growth and some behavior characteristics of Japanese quail during the whole of fattening period. The animal material of the study were total of 400 quails obtained from a mated randomly flock which was not previously genetically selected. There were significant differences between the groups according to the results of multivariate statistical analysis for weekly live weight of quail ($P < 0.05$). Considering all weeks, the mean values of live weights of the blue and green monochromatic lighting groups were determined to be higher than the red and white monochromatic groups. According to the results of the parallelism test, the difference between the groups was observed from one week of age ($P < 0.05$). The growth curves of groups were compared using the Gompertz function via non-linear regression analysis. The significant differences among the groups for the behavior characteristics of quail were found in terms of feed consumption, walking, standing, and drinking characteristics according to the time budget usage averages (all $P < 0.05$). As a result, it is possible to say that different monochromatic lighting applications affect the growth and some behavioral characteristics in Japanese quail.

Keywords: Monochromatic lighting, Growth curve, Behavior, Gompertz

P23 Usage Possibilities of Some Insect Species in Feeds

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Abstract

Usage Possibilities of Some Insect Species in Feeds In this study, basic nutrient contents and fatty acid methyl ester composition of larvae period (*Tenebrio molitor*), buffalo worm (*Alphitobius diaperinus*) and morio worm (*Zophobas morio*) were investigated by gas chromatographic methods and their possibilities of using in feeds were investigated. The highest crude protein ratio was found to be (as) 63.94% in the buffalo. The highest fat content was found in morio (39.99%). Crude fiber rates varied between 7.07-7.89%. The flour used in the study was dry, and the palmitic acid values of morio and buffalo worms ranged from 30.453 to 40.437%. Oleic acid values were found to be (as) 36.252-50.545%. Linoleic acid was found to be the highest value with 20.349% of buffalo. It is thought that dried insect larvae of used in the study can be used instead of animal protein sources when evaluated according to the nutrient parameters examined. However, detection of possible anti nutritive effects and performance values should be considered in further studies.

Keywords: Mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), morio worm (*Zoophobus morio*), feeds

P24 Investigation of Antibacterial Efficiency of Herbal Liquid Extract Mix as Natural Food Preservative against *Salmonella enterica subsp. enterica serovar Typhimurium*

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Abstract

In this study, we investigated the inhibitory effect of chicken fillet meat on the Salmonella bacterial population by treatment with Asatim® herbal liquid extract mixture (*Rumex acetocella*, *Achillea millefolium*, *Plantago lanceolata*- liquid extract mixtures) In the study chicken fillet meats used were divided into 2 groups and the first group was used as a control sample. The other experimental group was artificially contaminated with *Salmonella enterica* and treated with 1mL of Asatim® herbal liquid extract mixtures at approximately 10^8 kob/mL levels according to McFarland 0.5 equivalent.

Both groups were incubated at + 4°C for 24 hours. After incubation, the control group and the contaminated raw chicken fillet meat was performed according to the method Detection of Salmonella spp. ISO 6579-1:2017 Asatim® herbal liquid extract mixtures used in the study, detection of chlorine, detection of ethyl alcohol, detection of sorbic-benzoic acid analyzes were performed and no chemicals were found.

At the end of the study, in the control group was detected Salmonella spp., in the group treated with Asatim® herbal liquid extract mixture was not detected Salmonella spp. In this study, it is concluded that the herbal extracts of the plants in the flora of our country can be used as natural food preservatives in order to control and prevent Salmonella spp. contamination.

Keywords: Herbal extracts, *Salmonella enterica*, ISO 6579-1, *Rumex acetocella*, *Achillea millefolium*, *Plantago lanceolata*

P25 Chicken and Chicken Products in Turkish Food Culture

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Abstract

Recently, chicken meat is the most consumed meat all over the world regardless of religion, race and ethnicity. The Turks lived in the geographies the first domesticated of chicken are one of the first communities used to the chicken in their daily diet and they have been living together approximately for 4000 years. During this time, although Turks are generally known as animal husbandry community, chicken meat stayed in shadow of other meat types. Nevertheless, it is stated that chicken meat is processed and consumed with similar methods applied to red meat in historical references.

In this study, it was examined to deal with information about processing and utilization of the chicken meat in the written sources and travel books belonging to Turkish communities in chronological order.

P[26] Risk of Dioxin in Poultry Meat and Products

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Abstract

The term dioxin is used for all toxic forms of dioxin and dioxin-like compounds (polychlorinated phenyls, furans, brominated substances). Depending on environmental factors, poultry may take dioxins into their bodies, and dioxins can accumulate in poultry meat and products at high levels, mainly egg and liver. Dioxin is highly resistant to degredation in poultry metabolism and is distributed to tissues, transported to products derived from poultry and therefore poses a threat to public health. Through the measures taken over the past decade, dioxin emissions have been reduced and consequently dioxin levels in the environment, feed and food have begun to decrease. However it will take many years for dioxin concentrations to fall below the risk levels because of dioxins are slowly metabolized and eliminated due to being a highly persistent compound.

Keywords: poultry meat, egg, dioxin, residue, contaminant

P[27] Viable But Nonculturable *Salmonella* Problem in Poultry Industry

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Abstract

Nowadays, it is known that many pathogen microorganisms enter into a physiological state in which their cells are alive but cannot be produced in standard laboratory techniques, when they are exposed to various environmental stresses. The fact that microorganisms transform into Viable But Nonculturable (VBNC) state is considered as a survival strategy. Various environmental stress factors have been reported that trigger the transition of microorganisms to VBNC. It has been shown that VBNC microorganisms are morphologically smaller but can perform the synthesis of macromolecules. The point where the issue poses a risk for food industry is that VBNC microorganisms can regain their ability to reproduce and cause infections by their resuscitation. In food model systems, the presence of VBNC microorganisms in food after the resuscitation is shown as evidence of virulence. *Salmonella* serotypes, which are of great importance in terms of public health for poultry industry, have also been transform towards VBNC form throughout the food chain. Disinfection processes and storage conditions are reported to be quite effective in terms of resuscitation of VBNC *Salmonella* in food model systems. According to the relevant disinfection studies, in the results of analysis of target microorganisms based on traditional culture techniques, it was shown that the lack of identification of VBNC microorganisms could lead to mis-evaluation of the effectiveness of sanitation. On the other hand, in the case of all microorganisms are VBNC in the samples, food-borne infections can be attributed to viruses as conventional culture techniques do not detect a cultureable microorganism, which is a major problem for public health.

P28 Control of *Salmonella* Typhimurium and *Salmonella* Enteritidis with Peroxyacetic Acid Applied to Chicken Neck Skin

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Abstract

In this study, *Salmonella* control and decontamination levels on chicken meat were determined by using peroxyacetic acid (PAA) treatment on chicken neck skin samples. In this context, following the removal of the internal organs after the slaughtering, neck skin samples taken from the slaughter line, and they were contaminated with concentrations of 10^3 and 10^5 cfu/ml of *S*. Typhimurium and *S*. Enteritidis isolates. Following the attachment of the microorganisms to the neck skin samples, they were divided into groups for PAA treatments. Groups were classified as; 100 ppm PAA treatment for 10 seconds (group A) and 30 seconds (group B); 200 ppm PAA solution for 10 seconds (group C) and 30 seconds (group D). The PAA treatment concentrations were determined by taking the EFSA recommendations into consideration. The control (K) group was also classified to determine the attachment levels of the isolates without any decontamination treatments. In addition, neck skin samples treated with only PAA concentrations that were not contaminated with Salmonella isolates, were also classified as pH groups. All samples were kept at 4°C during shelf life. Microbiological and chemical analysis (pH) of samples were performed on the 0th hour, 6th hour, 1st day, 3rd and 5th days. When the results of the study are considered, it is observed that the PAA solutions do not change the pH of the neck skins at the applied concentrations. According to the results of microbiological analysis within the control of S. Typhimurium and S. Enteritidis on chicken neck skin samples, one logarithm reduction was achieved in the 6th hour after PAA treatments, but after 6 hours of the contamination, the microorganisms continued to grow gradually. This indicates that the treatment of 100 and 200 ppm PAA concentrations for 10 and 30 sec. was insufficient in terms of S. Typhimurium and S. Enteritidis decontamination on chicken neck skin samples.

Keywords: Chicken neck skin, *Salmonella,* peroxyacetic acid, decontamination

P[29] Natural Water Sterilisation at Broiler Farms for Removing Biofilm by Using Ultrasonic Techonology

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Abstract

The use of chlorine is very common in cleaning the areas where chickens are fed and meet their water needs. However, this application does not perform a high quality disinfection process. Biofilm formation continues in these regions. Biofilm is a perfect feeding and breeding ground for bacteria and viruses. Therefore, the use of antibiotics is necessary to protect animals from diseases caused by pollution therein. The use of antibiotics is a negative situation in terms of animal health and cost. For this reason, the idea of using low-energy ultrasonic waves has emerged in poultry farms or in areas with potential for biofilm formation and in water, to carry out the disinfection process. In this paper, the effect of ultrasonic waves on eliminating biofilm will be discussed.

P[30] Identification of *Salmonella* Gallinarum and Pullorum by qPCR

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Abstract

In this study, the DNAs obtained from 10 samples from the International Qualification-Comparison Tests with reference strains and VKMAE Poultry Diseases Laboratory were used in quantitative polymerase chain reaction (qPCR) studies and their applicability was evaluated. A rapid diagnostic kit was developed by performing QPCR with simultaneous *S*. Gallinarum/ Pullorum targeted reactions in the FAM channel. With this rapid diagnostic kit, it was seen that specific biovars could be distinguished. Since the ease of use, reliability, technically simple, full automation, specific, sensitive and fast, the qPCR method is thought to provide more advantages in the diagnosis and separation of these biovars.

P31 Investigation of *Salmonella* Serotypes in Routine Specimens Sent from Broiler Houses between 2013-2018

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Abstract

Salmonella serotypes that are scattered in the environment by the feces of infected animals are transmitted to the sensitive animals. Beside this, poultry meat and eggs are the most important source of *Salmonella* infections in humans. In this study, the presence of *Salmonella* serotypes in 1382 poultry dust and litter materials which were sent to our laboratory from broiler farms in 2013-2018 were investigated. Among the samples isolated according to the ISO6579 and serotyped according to Kauffmann-White scheme, 52.46% *Salmonella* positivity was determined. The most isolated serotypes were found to be *S*. Infantis (28.55%), *S*. Enteritidis (25.24%), *S*. Kentucky (9.24%), *S*. Mbandaka (4.68%) and *S*. Senftenberg (4.41%), respectively. According to these results, *S*. Infantis was found to be the most isolated serotype and overall isolation rate of *Salmonella* was found to be quite high according to EFSA 2017 report (3.31%).

P[32] Feed Enzymes, As Efficient Feed Additive for Balanced Gut Microbiota in Poultry

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Abstract

Exogenous enzymes, one of the feed additives, are the compounds that can contribute to the development of beneficial microorganisms in the digestive tract by providing maximum benefit from the nutrients and have negative effects on the growth of pathogenic microorganisms. Enzymes are particularly common in the evaluation of carbohydrates which are important components of poultry rations. Carbohydrates include mainly digestible nutrients such as starch and sugar, but also have low digestible nutrients such as cellulose, hemicellulose, pectin, beta glucan and lignin. For this reason, enzymes are very important in evaluating the components with low digestibility in the structure of feed and in reducing the negative effects of antinutrients on the intestine. In summary, enzymes have been one of the most important additives to improve performance and after the prohibition of antibiotics in the intestinal health.

P33 Supplemental Dietary Methionine Sources Have a Neutral Impact on Oxidative Status in Broiler Chickens

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Abstract

Methionine is an essential amino acid for poultry and the first limiting in broiler diets. Methionine is not essential only for protein synthesis and muscle deposition, but for several important biological functions related to oxidative stress. As a precursor for cysteine and subsequently glutathione (GSH). Methionine plays a very important role in the antioxidant system. The GSH system is essential to the preventative control of such oxidants and amelioration of oxidative stress (Gilbert 1984, 1985; Chai et al. 1994). Methionine is converted through the transsulfuration pathway to cysteine and further GSH. However, there are several isoforms of dietary methionine available that may affect the production of GSH and therefore the antioxidant capacity of tissues. For that reason, the current trial was designed to test the effects of various dietary methionine sources (DL-Met, L-Met or DL-HMTBA) on the GSH system and the balance between oxidative stress biomarkers and antioxidant capacity of tissues in broiler chickens. In conclusion the study shows that DL-Methionine, L-Methionine and DL-HMTBA (formulated at 65 % BE) did not differentially affect growth performance or the oxidative status in the digestive, metabolic and meat tissues of healthy broilers.

P[34] Effect of Bacillus Amyloliquefaciens CECT 5940 on Health Status and Live Performance in Broilers

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Abstract

The objective of this trial was to determine the efficacy of feeding *Bacillus amyloliquefaciens* CECT 5940 (Ecobiol®) on broiler performance. Three hundred Ross 308 males were randomly distributed in two treatments; basal diet (Control) and basal diet supplemented with *B. amyloliquefaciens* CECT 5940 (1.0×10^6 cfu/g of feed). Body weight gain was numerically higher in birds fed Ecobiol® compared to the unsupplemented group. Feed conversion ratio was significantly lower in the probiotic group at day 20 (P=0.003) and at day 35 (P=0.037). The results demonstrate that Ecobiol® is able to improve efficiency in high performing birds.

Key words: broiler, gut-health, performance, probiotic

P35 Effect of a Protease in Maize-Soybean Meal Diets on Broiler's Productive Parameters

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Abstract

In the last decades, the use of exogenous enzymes in animal feeding has become increasingly important. The use of enzymes may suppress the animals' deficiencies in endogenous enzymes, and may increase performance by improving protein digestibility. Maize and soybean meal (SBM) diets may contain high protein levels which nitrogen utilization may be enhanced by enzyme supplementation.

Therefore, the aim of the present study was to determine the effect of protease supplementation on maize and SBM diets in the productive parameters of broilers from 0 to 35 days of age. For this, 200 male chicks of the ROSS 308 breed were divided by four treatments, each with 10 replicates. Treatments consisted of a basal control diet (CD), a reduced protein diet that provided only 95% of crude protein and amino acids of the control diet (95CP), a reduced protein diet to which 130 mg/kg of protease was added (95CP+EP) and a reduced protein diet corresponding to the protease contribution in crude protein and amino acids to which 130 mg/kg of protease was added (CD-EP)+EP. The body weight (BW) and feed intake (FI) were measured weekly plus at feeding phase change (day 12 and 25). At the end of the trial, the dimensions of the digestive system's organs and the viscosity of the digestive contents were determined. In the first three weeks of the assay there weren't significant differences between treatments for BW and weight gain (WG). From the third week until the end of the trial, protease supplementation improved broiler's BW and WG subjected to low protein and amino acid diets, to make up the lack of nutrients.

The results indicate that this enzyme may have a positive effect on the broiler's productive parameters fed with maize and SBM diets, especially in low protein and low amino acid levels.

Key words: Broilers; maize; soybean meal; protease; protein

Introduction

Currently, chicken meat is one of the most consumed meat in the world, due to its good nutritional characteristics and at a reduced price compared to other meats. The fact that it is a low-fat white meat makes it one of the first choices for consumers, who are increasingly concerned about their health. The advances achieved in the area of nutrition, health, management and genetics have allowed to improve the animal, as well as produce high quality diets at a lower price. Thus, it enables the production of a product in a faster and efficient way, at highly competitive prices.

Maize and SBM are the most widely used ingredients in poultry diets. Maize has a higher energy value and a lower content of non-starch polysaccharides (NSP) when compared to other cereals, justifying its use in the broiler's feeding. The SBM is used because of its high protein value and low degree of lignification in relation to other plant proteins. SBM also contains antinutritional factors (ANF), which are protease and trypsin inhibitors, although part is destroyed during soybean meal's processing.

With protease supplementation it is possible to increase the concentration of enzymes capable of digesting protein, improving protein digestibility and nitrogen utilization by the animal, and consequently, an improvement in the broiler's growth.

The combined effect of the protease with other enzyme supplements has been extensively studied over the years. However, the effect of protease utilization as the sole enzyme supplement in maize and SBM diets on broiler production has been less studied. Thus, the objective of this work was to analyze the effect of protease's incorporation on the productive indexes of broiler chickens when fed a diet based on maize and soybean meal.

Materials and Methods

A total of 200 (1-day-old) Ross 308 broiler chicks were acquired from a local commercial hatchery. Chicks were reared under standard management practices of the species. All chicks were kept on broiler starter ration up to 12 days of age, changed to a broiler grower ration until 25 days of age and then fed a broiler finisher until the end of the trial (day 35) (Table 1). In the phase changes one broiler was removed per cage. The feed and water were provided *ad libitum*. The temperature was maintained at 28-29ºC during all time of the trial. Lighting was provided for 24 hours throughout the experimental period.

The chicks (n=200) were placed 5 per pen with 10 pens per treatment. Treatments were randomized within blocks. Treatments consisted of a basal control diet, a reduced protein diet that provided only 95% of crude protein and amino acids of the control diet, a reduced protein diet to which 130 mg/kg of protease was added and a reduced protein diet corresponding to the nutritional up-lift advised by the protease supplier to which 130 mg/kg of protease was added.

Table 1. Ingredient and nutrient composition of the experimental diets

Item	CD			95CP and 95CP+EP			(CD-EP)+EP		
Ingredient, %	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
Maize	63,400	64,750	70,445	66,255	67,428	72,870	64,812	66,258	71,845
Soybean meal, 47%	30,854	23,056	18,174	28,392	20,677	16,11	29,655	22,210	17,000
Extruded soybean	0	7,54	7,00	0	7,61	7,00	0	7,00	7,00
Soybean oil	1,205	0,990	1,220	0,820	0,605	0,882	1,000	0,850	1,020
DL-Methionine	0,381	0,312	0,250	0,360	0,300	0,235	0,377	0,312	0,245
L-Lysine	0,335	0,230	0,260	0,330	0,235	0,263	0,326	0,230	0,260
Calcium carbonate	1,230	1,057	0,970	1,233	1,060	0,970	1,235	1,060	0,970
Dicalcium phosphate	1,945	1,435	1,070	1,960	1,455	1,060	1,945	1,450	1,050
Premix[1]	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3
Salt	0,350	0,330	0,311	0,350	0,330	0,310	0,350	0,330	0,310
Formulated nutrient composition, % unless noted									
AME, kcal/kg	3050	3130	3200	3050	3131	3201	3050	3130	3200
DM	84,12	84,86	85,28	84,00	84,78	85,19	84,04	84,83	85,24
CP	20,40	19,20	17,00	19,39	18,24	16,16	19,92	18,74	16,52
Lysine	1,22	1,11	1,00	1,16	1,06	0,95	1,19	1,08	0,96
Methionine	0,68	0,61	0,52	0,65	0,58	0,49	0,67	0,59	0,51
Ca	0,95	0,78	0,65	0,95	0,78	0,65	0,95	0,78	0,65
P	0,47	0,38	0,30	0,47	0,38	0,31	0,47	0,38	0,30

[1]The premix supplied per kilogram of diet: Pantothenic acid 10 mg, vitamin D3 2400 IU, cyanocobalamin 0,02 mg, folic acid 1 mg, vitamin K3 2 mg, niacin 25 mg; vitamin B6 2 mg, vitamin A 10000 UI, vitamin B1 2 mg, vitamin E 30 mg, vitamin B2 4 mg, Cu 8 mg, Fe 50 mg, I 0,7 mg, Mn 60 mg, Se 0,18 mg, Zn 40 mg; ethoxyquin 1,5 mg; 6-phytase EC 3.1.3.26 1500000 FYT.

All the chicks underwent a pre-selection process, they couldn't have diseases or injuries and weighing between 45 and 55 grams. With this, they were arranged in the cages randomly. The FI, BW and feed conversion ratio were measured. After the trial, one bird per cage was euthanized. The digestive contents of the duodenum and jejunum and of the ileum were collected for centrifugation tubes to measure the viscosities. Also, we measured gut, gizzard, pancreas, liver, duodenum, jejunum, ileum and cecum, and measured the length of the duodenum, jejunum, ileum and cecum. In addition, samples of all treatments and all diets were taken to calculate crude energy, dry matter, ash, crude protein, crude fiber, crude fat and ether extract.

Statistical analysis was performed using the analysis of variance using the General Linear Models procedure of the statistical program SAS (SAS, 2001). Mean values with significant F value ($P < 0.05$) were compared using the Duncan test. Differences between means were considered significant when $P < 0.05$.

Results and Discussion

By observing the obtained values at Table 2, it's verified that at day 0, the chick's BW distributed by the four treatments didn't differ, and there was no initial advantage for any of the treatments. At days 7 and 14, the BW of the chicks remains similar. At day 28, the BW of broilers fed (CD-EP)+EP was higher ($P < 0.05$) than the 95CP treatment, but didn't differ from the results obtained for the animals subjected to CD and 95CP+EP treatments. At days 21 and 35, the broiler's BW submitted to treatment (CD-EP)+EP was similar to the other treatments. Between days 21 and 28, diets deficient in 5% crude protein and amino acids had a significant decrease in performance, which was corrected with the protease addition in the last week of the trial. In the starter phase, there were no differences between the four treatments (P> 0.05). Already during the grower phase and the finisher phase, it was observed that broilers subjected to treatments with crude protein and amino acid deficits, had significant performance´s losses in relation to the control diet that were corrected with protease enzyme supplementation. In the present study, the obtained results demonstrate that the protease used was sufficient to improve protein digestibility. These results are in agreement with another study, where the addition of amylase, amyloglucanase and protease to a maize and SBM diet didn't lead to significant differences between treatments in the first two weeks of the experiment [1].

Table 2- Effects of Enzyme Protease on productive parameters, organ's dimensions and viscosity

MEASUREMENT	CD	95CP	95CP+EP	(CD-EP)+EP	SEM	p(F)
0 d BW, g	48,4	48,9	48,0	48,5	0,200	0,466
7 d BW, g	201,1	201,7	203,4	202,5	1,088	0,896
12 d BW, g	389,2	388,4	391,9	394,4	2,350	0,797
14 d BW, g	498,0	487,8	491,7	494,4	3,071	0,693
21 d BW, g	947,5 ^{a}	898,3 ^{b}	914,6 ^{ab}	929,3 ^{ab}	6,386	0,042
25 d BW, g	1259,8 ^{a}	1187,3 ^{b}	1209,8 ^{ab}	1235,1 ^{ab}	8,924	0,024
28 d BW, g	1507,6 ^{a}	1409,3 ^{b}	1441,8 ^{ab}	1481,9 ^{a}	11,973	0,018
35 d BW, g	2095,6 ^{a}	1995,5 ^{b}	2014,1 ^{ab}	2083,1 ^{ab}	15,224	0,044
0-12 d WG, g	28,4	28,3	28,7	28,8	0,192	0,752
12-25 d WG, g	67,0 ^{a}	61,5 ^{b}	62,9 ^{b}	64,7 ^{ab}	0,618	0,010
25-35 d WG, g	83,6	80,1	80,6	84,8	0,905	0,190
0-35 d WG, g	58,5 ^{a}	55,6 ^{b}	56,2 ^{ab}	58,1 ^{a}	0,435	0,043
0-12 d FI, g	456,5	462,6	467,2	467,2	2,500	0,391
12-25 d FI, g	1235,4	1227,4	1236,7	1277,4	14,209	0,614
25-35 d FI, g	1523,9	1542,2	1525,6	1552,5	17,657	0,935
0-35 d FI, g	3215,8	3232,1	3229,5	3297,2	29,311	0,778
0-12 d g/g	1,34	1,36	1,36	1,35	0,011	0,898
12-25 d g/g	1,42 ^{b}	1,54 ^{a}	1,52 ^{a}	1,52 ^{a}	0,017	0,048
25-35 d g/g	1,83 ^{c}	1,91 ^{a}	1,89 ^{ab}	1,84 ^{bc}	0,012	0,027
0-35 d g/g	1,57 ^{b}	1,66 ^{a}	1,64 ^{a}	1,62 ^{ab}	0,011	0,016
35 d Crop, g/kg BW	2,15	2,08	2,17	2,19	0,062	0,934
35 d Gizzard, g/kg BW	12,50	11,91	12,02	11,66	0,190	0,480
35 d Pancreas, g/kg BW	2,27^{a}	2,08^{ab}	1,86^{b}	2,03^{ab}	0,054	0,053
35 d Liver, g/kg BW	23,14	24,69	25,07	25,96	0,522	0,287
35 d Duodenum, g/kg BW	4,87	4,54	4,59	4,90	0,097	0,438
35 d Jejunum, g/kg BW	8,80	8,63	8,82	8,99	0,176	0,918
35 d Ileum, g/kg BW	7,92	7,86	7,14	7,98	0,230	0,556
35 d Cecum, g/kg BW	3,14	3,31	3,02	3,63	0,114	0,263
35 d Duodenum, cm/kg BW	13,46	13,98	12,97	13,74	0,229	0,451
35 d Jejunum, cm/kg BW	30,87	34,99	33,26	33,97	0,643	0,131
35 d Ileum, cm/kg BW	32,94	33,72	33,04	34,26	0,552	0,825
35 d Cecum, cm/kg BW	7,28	7,51	7,73	7,59	0,187	0,863
35 d Duoden+Jejun, cpo	6,35	5,19	5,29	5,28	0,322	0,543
35 d Ileum, cpo	4,02	3,83	3,96	3,57	0,183	0,838

a,b,c Means within rows with different superscripts differ significantly (P < 0.05).

On another occasion, a study found that during the day 1 to 22 a low energy and mineral diet supplemented with an enzyme mixture containing protease had the same BW as the non-supplemented control diet [2]. It was also verified previously that with a maize and SBM diet, treatments supplemented with xylanase, amylase and protease had better BW at day 16 and day 35 [3]. Toledo et al. found that a diet with reduced levels of energy, crude protein, and amino acids supplemented with xylanase, β-glucanase, cellulose, pectinase, and protease had lower BW levels than normal diet at day 1 unprocessed crude protein and amino acids [4].

Regarding the values obtained for the WG, at the starter and finisher phase, there were no significant differences between treatments. In the grower phase, there were significant differences between the CD and the 95CP and 95CP+EP treatments. From the results obtained, it is possible to state that the supplemented protease was satisfactory to improve WG by the animal. These results are also in agreement with the study where the supply of amylase, amyloglucanase and protease to a maize and SBM diet did not lead to significant differences between treatments in the first two weeks [1]. Cowieson et al. obtained broilers with higher WG when fed maize and SBM diets with low energy and amino acid levels supplemented with xylanase, amylase and protease in relation to the conventional diet [5]. With maize and SBM diets supplemented with xylanase, amylase and protease, a comparison was made between a conventional diet to one supplemented with xylanase, amylase and protease, where the energy content corresponding to the contribution of the enzyme mixture was reduced. There were no significant differences between treatments, showing that the improvement in nutrient utilization by enzyme supplementation completely compensated for the reduction in energy content [6]. It is important to stress, however, that the previous studies described here used for comparison purposes, used an enzyme cocktail that included protease rather than protease alone such as in our study.

In the present study, feed intake was not different between treatments throughout the entire study. These results are in agreement with other studies [1,5,7], where for a maize and SBM diet, a control diet and another low energy density and crude protein supplemented enzymatically there were no significant differences in feed intake between the treatments. The improvement in animal performance observed was due to changes in energy and amino acid's digestibility rather than a better intake of digestible nutrients. These speculations are supported by the improvement of apparent metabolizable energy, nitrogen retention and amino acid digestibility.

Yu et al. (2007) found that supplementation of protease and with/without carbohydrase in a low-protein diet during the starter phase had lower intakes than a non-supplemented diet in the hot season. The author explains that it may be due to increased protein digestibility and amino acid's availability due to enzymatic supplementation during the warm season.
At the starter phase, there were no significant differences in feed conversion ratios between treatments ($P > 0.05$). In the grower and finisher phase, broilers subjected to the 95CP and 95CP+EP had higher FC ($P < 0.05$) compared to the CD. However, broilers undergoing treatment (CD-EP)+EP had higher FC ($P < 0.05$) compared to CD in the grower phase, but recovered performance in the finishing phase. Due to improvement in the finisher phase, overall there weren't significant differences between the treatments (CD-EP)+EP and the CD. Many authors had found no significant differences between control diets and low density diets supplemented with protease or enzyme mixtures [1,4,6,8]. Angel demonstrated that a maize and SBM diet with a reduced protein content supplemented at 200 mg/kg protease, presented similar FC to non-supplemented control diet [9].

At the end of the trial, there were no significant differences between treatments for the relative weight of the gizzard, liver, duodenum, jejunum, ileum and cecum ($P > 0.05$). In relation to the relative length of the duodenum, jejunum, ileum and cecum, there were no differences between treatments ($P > 0.05$). The relative size and weight of the digestive tract organs is reflected in the carcass yield, and therefore, the lower the values, the better the carcass yield, a parameter of great economic interest to poultry's farmers. According to [10], the increase in organ's weight and length is due to high viscosity, which inhibits contact between digestive enzymes and their substrates, leading to structural and functional changes in the intestine. Brenes indicate that this increase in the size of organs of the digestive system may be an adaptive response to an increasing need for enzymes [11]. For this study, there were no significant differences between the relative weight and length of the broiler's organs in the different treatments. The absence of significant differences in the size of the organs may be due to the low amount of soluble NSP in maize and SBM, and a change in the digestive system organ's size is not expected. These results are also in agreement with [2,6], that showed that there were no significant differences between treatments for organ dimensions. In the present study, it was a found a heavier pancreas in broilers in the CD treatment in comparison to broilers in the 95CP+EP treatment, indicating a potential reduction in the production of pancreatic enzymes due to the effect of the exogenous proteolytic enzyme that was added.

The viscosity of the digestive contents is a very relevant problem for barley or rye-based diets and less for the wheat-based diets based, being minimal in the case of maize. This is due to the type of soluble fibers and the size of their chains being much smaller in the case of maize than in the other cereal grains. Knudsen showed that maize contains low concentrations of soluble NSP (<1 g / kg vs 25 g / kg in wheat), so issues such as viscosity, flow rate, water retention capacity are not considered relevant in the case of maize [12]. However, the nutritional value of maize varies considerably, despite having a low concentration of soluble PNA, it is considered to be due to the solubility / digestibility variation of the starch and protein [5]. [13] explains that the starch's solubilization occurs during the granulation process of the food but that the magnitude of its impact is so minimal that the effect is likely to be of little importance. Regarding the values obtained for the viscosity, there were no significant differences between treatments for the duodenum+jejunum and ileum contents at the end of the trial. The reason why there were no significant differences is due to the fact that the protease does not act on the NSP. These results are also in agreement with the studies carried out by Zanella et al., and Knudsen [6,12], which supplemented a maize and SBM diet with α-amylase and did not obtain significant differences between treatments [12]. Also [6], in a maize and soybean diet, where the treatments present different sources of soybean (toasted, extruded and meal) supplemented with xylanase, amylase and protease also did not obtain significant differences between treatments.

Conclusion

For low protein and amino acid diets, protease supplementation improved body weight and weight gain in order to compensate for the lack of nutrients. The treatment in which the contribution of the crude protein and amino acids by the protease was withdrawn, it had a similar feed conversion to the control diet in the finisher phase, compensating the loss of performance in the grower phase. This compensation allowed, in general, that both treatments had a similar performance. There were no significant differences between treatments for feed intake, viscosity of the digestive contents and dimensions of the organs of the digestive system, with the exception of the pancreas. These results allowed to conclude that protease enzyme supplementation improved the productive parameters, especially in low protein and amino acids diets. It can also be said that protease supplementation may be used to reduce the levels of crude protein and amino acids in a diet without changing the broiler's productive parameters, allowing to reduce the nitrogen's excretion and environmental impacts.

This work contributed to reinforce the positive effect of protease supplementation on the broiler's productive parameters in maize and soybean meal diets. Still, more work should be done with protease, as the only enzyme supplement, in order to establish its true potential in the poultry sector.

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P[36] Effect of Egg Turning Frequency during Incubation on Hatchability (Field Study)

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Abstract

The field study was conducted that the effects of increasing the frequency in the first week of incubation on hatchability and second grade chick percentage in a commercial hatchery. It was planned two different experiments in the study, where hatching eggs were collected from Ross 308 commercial broiler breeder flocks. In the first Experiment, eggs were turned 24X/day during the first 14 d of incubation, and eggs were not turned during 15-18 d of incubation. In the other machine, eggs were turned 96X/day for the first 7 day, 24X/day from 8 to 14 days, and no turned during 15-18 d of incubation. The 2 machines with eggs were turned 96X/day either during 3 d or during 7 d in Experiment 2, and eggs were turned 24X/day until 14 d of incubation. It was determined that increased turning frequency in the first week caused a positive effect on the hatchability in 21 of 24 incubation batches in Experiment 1 ($P<0.05$). However, there was no significant difference among groups in Experiment 2 ($P>0.05$). There was no significant difference in second grade chick percentage in both experiments. Consequently, these data demonstrated that hatchability was increased owing to 96X/day turning instead of 24X/day during the first week of incubation. Moreover, the frequency of turning in the first 3 d or 7 d had a similar effect on the hatchability, so 96X/day turning during the first 3 d may be helped to increase the hatchability.

Key word: Turning frequency, hatchability, second grade chick percentage

P[37] The Effect of Egg Position and Chick Pipping Location on Hatchability and Broiler Live Performance

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Abstract

This study was carried out to determine the effects of egg position and chick pipping location on hatchability and broiler performance. A total of 1350 eggs collected from the Ross 308 commercial broiler breeder flock at 38 wk of age. Before the incubation, the location of the air space of each egg was controlled and divided into two groups, where the eggs incubated either with their large ends (LEU) or small ends up (SEU). On the 18.5 d of incubation, the eggs were transferred to the hatcher trays, and the air space area was checked and marked on the egg surface, so chick pip location was determined. The locations were air space (AS), middle (ML), and small ends (SE) of the eggs. Hatchability, embryonic mortality, and malpositioned embryo of fertile eggs were calculated, and hatching time of groups was determined. A total of 864 chicks were pulled, weighed and transferred to floor pens. Body weight, feed conversion ratio, livability, and European Production Efficiency Index (EPEI) were evaluated. Chicks were individually weighed at 0, 7 and 35 d. Feed conversion ratio and livability were calculated for 0-7 d and 0-35 d period. Z-Test was employed to determine the existence of differences between two proportional values of hatchability and embryonic mortality for the groups. Broiler performance of the groups was analyzed using ANOVA procedure. Although all of the 450 LEU eggs pipped in the AS location, 10% of 900 eggs in the SEU position. It was determined that eggs incubated in the SEU position caused a significantly decrease in fertile hatchability because of increased late embryonic mortality, especially malpositioned embryo ($P<0.05$). In addition, SEU position had a longer hatching time. It was determined that chick BW was significantly affected by egg position and pipping location ($P<0.05$), and chicks hatched from SE had the lowest BW. The highest BW was observed in LEU group, while SE had the lowest BW. At 35 d, there was no significant difference in BW, FCR, and livability, but EPEI was decreased due to SEU egg position ($P<0.05$). As a result, it was determined that the errors of egg placement caused a negative effect on hatchability and broiler performance.

Keywords: Egg; Position; Incubation; Broiler; Performance

P38 In Ovo Injection Aplication in Broiler Breeders

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Abstract

In recent years, due to intensive selection in direction of growth rate in broiler breeders changed nutrient requirements and increased metabolic rate of embryos suggests that in ovo (IO) methods should be investigated. In ovo is a method based on injecting hormones, antioxidants, vitamins, minerals and various nutrients into the ideal area of fertile eggs at appropriate embryonic age. IO injection is applied to yolk, albumin and air cavity of egg at onset of incubation, and air cavity, yolk and allantoic sacs, embryo and amniotic fluid of the amniotic sac on days 1, 7, 13, 14, 17, 17.5, and 18 of embryonic age during incubation. Many studies have concluded that the injection of nutrients and other components to the egg by IO method increases the immune and digestive system efficiency, accelerates chick development, and positively affects many physiological parameters related to hormone and enzyme activity.

Key words: Embryo, broiler breeder, in ovo injection, in ovo application, in ovo feeding

P[39] Effects of Genotype and Floor Material on Broiler Welfare

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Abstract

The aim of this study was to evaluate the effects of genotype and floor material on broiler welfare. In the study, slow growing (Hubbard JA57) and fast growing (Ross 308) broilers with slatted floor and deep litter were used, so there were four main groups (2 genotype x 2 housing) and each main group was consisted of 5 replicates. Each replicates was consisted of 10 male chicks and 200 birds were used in total. The experiment was lasted for 8 weeks. The welfare parameters were recorded on week 8. Slow growing broiler had better welfare parameters than fast growing broilers. Slatted floor had a positive effect on main welfare parameters of the birds.

Key Words: Broiler, slow growing, slat floor housing, welfare, behavior.

P[40] Stilbenes and Usage of Stilbenes in Poultry Meat

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Abstract

Stilbenes are complex phytoalexins that are mostly found in plant species such as grapes, pine, peanuts and berries in the plant kingdom. They have numerous therapeutic effects on human body. Above all, they display antioxidant effects. Furthermore, they have additional important properties such as antimicrobial, anti-inflammatory, neuro-protective, anticancer, antimutagenic effects and also contributes to heart health. Poultry meats are very sensitive to microbial and oxidative deteriorations. These deteriorations lead to undesirable changes in poultry meats and this affects shelf life in a negative way. Various antioxidants and antimicrobial agents are used to prevent these problems. Nowadays, the rise in the tendency of consumers towards natural products has increased the demand for the use of natural preservatives and antioxidants in poultry meat. Stilbenes have great potential at this point due to their free radical scavenging activity and antimicrobial effects.

P41 Effect of Collagen Peptide Addition on Functional Characteristics of Chicken Meatballs

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Abstract

The effect of collagen peptide (CP) addition at 5%, 10%, 15% or 20% levels on some quality characteristics of chicken meatballs was evaluated. Chicken meatballs were analyzed for pH value, CIE lightness (L*), redness (a*) and yellowness (b*) color values, weight loss and diameter reduction after cooking, instrumental texture and sensory characteristics. Chicken meat balls with the addition of 20% CP possessed lower pH value and cooking loss in comparison to the control ($p<0.05$). All CP incorporated groups exhibited higher L* values than the control ($p<0.05$). Hardness value showed decreases with the addition of CP greater than 10% levels ($p<0.05$), whereas, gumminess and chewiness values decreased significantly with the addition of 20% KP ($p<0.05$). In sensory evaluation CP incorporated chicken meatballs rated acceptable in terms of all attributes tested. The data obtained from the present study suggest that CP could be used in chicken meat ball formulation as an alternative natural additive to develop new functional poultry meat products.

Keywords: Chicken meat, Collagen peptide, Functional product, Chicken meatballs

P[42] Importance of Turkey Meat, Properties and Turkey's Status

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Abstract

Animal protein sources have an important place for people to complete their physical and mental development in a healthy way. Poultry meats, which include turkey meat, are preferred instead of red meat in terms of health due to low cholesterol level and high protein / calorie ratio. Turkey meat is extremely healthy, nutritious and delicious. The protein content of turkey meat is around 20% and it contains many amino acids, especially lysine. Turkey meat is a very good source of B group vitamins and is also very rich in mineral substances. According to FAO 2017 data, poultry meat production is around 122 million tons, chicken meat 109 million and turkey meat 6 million tons. In Turkey, the production of poultry meat chicken meat production, according to data TÜİK from the year 2018 2.137 million tons, turkey meat production is about 52,363 tons. Turkey meat consumption is quite low compared to consumption in developed countries. Turkey meat is preferred by many people all over the world because it can be processed to various products and is delicious, it is seen as an alternative to red meat all over the world especially in terms of color and taste. It is necessary to encourage the consumption of this valuable animal food in our country not only at the beginning of the year, but also to inform the society about turkey meat, to introduce processed products, to support the growers and to increase production.

Keywords: Turkey Meat, healthy, nutritious

P43 Usage of Algae in Broiler Nutrition

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Abstract

Algae are single-cell microorganisms that can photosynthesis, live in single or colony in eukaryotic cell structure. Algae are divided into two groups as macro algae and micro algae. Macro algae reach very large sizes and cover coastal areas. Micro algae are small-sized organisms found in ocean waters, such as phytoplankton. They are divided into three groups as brown (*Phaeophyceae*), red (*Rhodophyceae*) and green (*Clorophyceae*) algae. Algae are used in the cosmetics, health, and energy sectors, as well as in the nutrition of humans and animals, and are used in the production of nutrients such as lipid, protein, carbohydrate, carotenodes. The use of algae in broiler chickens has been emphasized for years and studies have been conducted with different types of algae. Algae are used as feed additives for many different purposes in feeding broiler chickens. These vary according to the nutrient composition of the algae additive or the active substance it contains. Generally, the use of algae contributes to performance in broiler chickens and the products obtained from algae as stated above are intended to contribute to the feeding of broiler chickens. Studies have shown that the use of algae in broiler chickens contributes to performance improvement and increases the accumulation of components such as omega-3 in chicken meat. Food and water shortage due to the increasing population in the world, directs producers to alternative feed raw materials and feed additives in animal nutrition and algae become one of these additives. In this review, researches about the use of algae as feed additive in broiler feed were collected.

Key Words: Algae, feed additive, broiler chicken, feeding.

P[44] Usage Possibilities of Microalgae Species *Spirulina Platensis* in Poultry Diets

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Abstract

Spirulina platensis, which is cyanobacteria, is a crucial functional food additive. *Spirulina platensis* which is a photosynthetic micro algae, contains high crude protein content (62-70%) as well as precious metabolites. These proteins are mainly composed of essential amino acids. In line with the manufacture's demands, poultry producers use to natural, functional additives rather than synthetic additives. Studies on poultry have shown that Spirulina is a natural and functional nutrient. It was determined that Spirulina supports growth and development, increases fertility, improves carcase color and egg yolk color score, provides resistance to animal against diseases, and improves product quality. In this study were presented, the nutritional values of the microalgae species Spirulina were explained and the usability of the past and current sources and poultry rations. The basis for application as a poultry feed supplement is discussed.

Key words: Poultry feeding, Spirulina, Nutrient content, Functional feed additive.

P45 Effects of Arabinoxylan and Xylan Oligosaccharides on Performance and Gut Health in Broilers

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Abstract

Antibiotics have been widely used as growth promoters in poultry nutrition in order to optimize performance through improving intestinal health in previous decades. However, the use of antibiotics for growth promotion in poultry nutrition was banned due to concerns about development of antibiotic-resistant pathogens. Therefore, there has been ongoing research on potential antibiotic alternatives such as, probiotics, organic acids, plant extracts and prebiotics etc. in poultry nutrition. Prebiotics, particularly fructo-oligosaccharides and mannan-oligosaccharides, have been studied extensively and demonstrated that they have beneficial effects on intestinal health and growth performance of broiler chickens. Recently, in addition to aforementioned oligosaccharides, arabinoxylan and xylan oligosaccharides (AXOS and XOS) are getting attention for being potential antibiotic substitutes in broiler diets. AXOS and XOS, as feed additives, are obtained through partial hydrolysis of arabinoxylan and xylan polysaccharides, respectively. XOS are formed mainly by xylose units linked via β-(1–4) glycosidic bonds as AXOS are comprised of mixture of arabinose substituted XOS. The results of numerous *in vivo* research have been shown that AXOS and XOS pass undigested through the upper gastrointestinal tract and are then selectively fermented by beneficial microorganisms in the distal part of intestine. Selective fermentation of these oligosaccharides has been demonstrated to increase the number of *Lactobacillus* spp., *Bifidobacterium* spp., Clostridium cluster XIV and thereby stimulate production of lactic acid and short-chain fatty acid (SCFA), including butyrate, in the hindgut of broilers. Decreasing *Salmonella* Enteritidis counts, improving intestinal morphology and subsequently increasing growth performance have also been reported by some studies in broilers. In this respect, AXOS and XOS as prebiotic sources, could be included in broiler diets in order to contribute better intestinal health and consequently improve performance.

Key words: Arabinoxylan oligosaccharides, broiler, gut health, performance, xylan oligosaccharides

P[46] Lysolecithins in Broiler Nutrition

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Abstract

In recent years, genetic improvement in broiler breeding has changed the nutrient requirements and ration densities of broilers. In addition, genetic improvement reduced age of the slaughter, improved feed conservation and increased carcass breast and thigh meat ratio. Because of these developments, best utilization from the energy source was taken into consideration as well as all requirements of the broilers in order to realize fast development and to create economic rations. Energy requirement of broiler chickens is mostly met by the oil sources in the feed but especially enzyme insufficiency in young chicks has a negative effect on the utilization of these sources. In this scope, in order to increase the utilization of oil resources in feed, the addition of feed emulsifier to broiler feeds has on agenda in recent years. For this purpose, the use of lysolecithins, which are used as emulsifiers in the food industry, as feed additives, has been investigated and in this review, we aimed to evaluate the effects of lysolecithin usage on performance and nutrient digestibility in broiler feed.

Key Words: Broiler, lysolecithin, performance

P47 Effects of Lysine Source and Forms in Broiler Nutrition

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Abstract

In broiler nutrition, lysine is an essential aminoacid because it is both a reference amino acid and second limiting amino acid. Feed raw material used in broiler feed formation are insufficient in terms of lysine. Therefore, pure form of lysine should be added to broiler feeds. Lysine HCl (hydrochloride) has been widely used for this purpose in broiler nutrition. In addition, the use of lysine sulfate has been on the agenda in recent years. In this review, the effects of using these two lysine sources in broiler feed were evaluated.

Key Words: Broiler, lysine source, lysine sulphate, lysine hydrochloride

P48 Quinoa in the Production of Gluten-free Chicken Burger

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Abstract

Celiac, an immune system disease, is derived from small intestine inflammatory as a result of gluten consumption in gluten intolerant humans. The number of celiac patient in our country is 40703 according to the data reported in 2017; however, there is a great number of non-diagnostic celiac patient is considered. Gluten-free diet is inevitable for celiac patient in their whole life. In our country, there are various type of cereal products for celiac patients; however, there is not any gluten-free meat products in the market place. At this point, it is crucial to increase variety of gluten-free products in terms of improving life standard of celiac patient. With that said, production of gluten-free chicken burger using quinoa, which is a popular plant and suitable for the consumption of patient with celiac, was designed in this study.

Key words: Celiac, meat products, chicken burger, gluten-free diet, new product development

P49 Effects of Particle Size and Feed Form on Broiler Performance

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Abstract

In recent years, feed physical form and feed particle size have an important role in the health and performance of broiler, as well as in the breeding of a profitable broiler. Generally, feed, broiler chickens are given in two physical form in our country and also in the world. These are called mash feed and pellet feed. The use of pellet feed in the feeding of broilers in the light of the studies has positively affected the animal's digestive system, feed utilization, growth performance and digestibility of nutrients. Also, pellet feed, in addition to reducing feed loss, is also used in our country and in the world with its advantages in packaging and transportation. However, despite these advantages and some businesses are using mash feed. One of the biggest reasons for that the mash feed is cheaper than of pellet feed. In addition, some feed factories are expensive to install pelleting unit and therefore pellet feed unit does not contain, So they only produce mash feed**.** On the other hand, particle size increases while promoting gizzard development and the contact surface increases of fine grinding feed particles with digestive enzymes in the digestive channel. . The very finely ground feed materials did not have any positive effects on the digestive system but they have led to negative results. In this review, studies on the effect of feed physical form and feed particle size on broiler performance were collected.

Key Words: Feed form, feed particle size, broiler

P50 The Effects of Microwave, Sous Vide and Traditional Cooking Methods on Some Physicochemical and Textural Properties of Chicken Meat

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Abstract

Cooking method affects the physicochemical and sensory properties of meat. In this study, some physicochemical and textural properties of chicken meat were investigated by using traditional methods such as oven cooking, boiling and grilling and microwave, sous vide cooking method until the temperature of the center reached 76-77 ° C. It was found that the pH values of the samples ranged between 6.11 and 6.29 after cooking and the differences between the pH values were insignificant ($p > 0.05$). The cooking loss of chicken meat samples cooked by microwave method was 23.99% and the highest samples (35.56%) were determined in oven-baked samples. Texture of chicken meat samples with different cooking methods; Meullenet-Owens Razor Shear (MORS) and Warner Bratzler (WB) blades. The difference between the MORS results of the samples was statistically significant ($p < 0.05$); the lowest MORS values showed samples cooked by boiling. The difference between Warner Bratzler shear force (WBSF) and Warner Bratzler shear energy (WBSE) analysis results was insignificant ($p > 0.05$).

Keywords: Chicken meat, Cooking method, Microwave, Sous vide, Tenderness, Texture

P51 Metagenomic Analysis of Gut Microbiome Associated With *Salmonella* in Broilers

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Abstract

To determine gut microbiome of broiler chickens associated with *Salmonella*-colonization, samples of *Salmonella*-free and *Salmonella*-infected chickens were subjected to next generation sequencing and metagenomic analysis of bacterial 16SrDNA sequences. There was no significant difference between the bacterial diversity of 60 *Salmonella*-free and 60 -infected chickens from each 10 epidemiologic units (EU). *Firmicutes*, *Bacteroides* and *Proteobacteria* were the dominant phyla in both groups. Dominant families were *Bacteroidaceae*, *Ruminococcaceae* and *Lachnospiraceae* with 23.8, 12.3 and 11.3 per cent relative abundance in *Salmonella* free chickens; and *Bacteroidaceae*, *Enterobacteriaceae* and *Clostridiaceae* with 16.4, 9.3 and 4.8 per cent in *Salmonella* infected chickens. Dominant genera were *Bacteroides, Faecalibacterium* and *Butyrovibrio* with 20.3, 8.2 and 5.8 relative abundance in *Salmonella* free chickens; and *Bacteroides*, *Escherichia* and *Anaerohabdus* with 10.1, 5.4 and 4.5 per cent in *Salmonella* infected chickens. The relative abundance of butyrate producing bacteria was significantly higher in *Salmonella*-free samples. The microbiome containing higher levels of *Gammaproteobacteria*, *Enterobacteriaceae*, *Fusobacterium*, *Escherichia* and lower levels of *Bacillus*, *Lactococcus, Lactobacillus, Bifidobacterium* and butyrate producing bacteria were found to be associated with *Salmonella* occurrence.

P[52] Impact of Protected Benzoic Acid on *E. Coli, Salmonella* Colonization, Litter Quality and Pododermatitis in Broilers: A Field Trial Run Under Commercial Conditions

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Introduction

Antibiotics played a key role in reaching todays production levels, however health concerns have led to legislation and consumer pressure calling for livestock production to be less reliant on antibiotics and focused on natural feed additives. Organic acids (OA) are naturally occurring compounds with acidic and antimicrobial activities and have been used since very old time in preservation of foodstuff (Broom et al, 2016). OA have found to be effective and beneficial feed additives in filling the gap of modulation of gut microbiota after the ban of antibiotic growth promoters (AGPs) especially in EU and other follower countries. They have been shown to positively impact the gut microbiota, shifting the bacterial balance towards the beneficial bacteria e.g. *Lactobacillus* and reducing the harmful bacteria such as *E.coli, Salmonella* and *Clostridium.* Their positive effect on gut morphology and the immune system have already been documented. The form of administration of OA (acid, salt, protected or adsorbed on a mineral carrier) determine the efficacy for its intended purpose. Typically, the protected OA have the most observable effect upon gut health (Partanen and Mroz, 2012; Dibner and Butin, 2002; Fascina et al., 2017). OA tend to alter the gut ecosystem in monogastric animals by reducing pH in the stomach and gut and suppressing harmful bacteria through their bacteriostatic effect (Choct, 2009). Growth and feed efficiency have been also reported to be improved (Özdüven et al., 2009; Sabour et al, 2018).

Concerning their mode of action, it has been reported that OA can easily diffuse through semi-permeable membrane of the bacteria into the cell system when they are in the undissociated form. Organic acids are weak acids and only partly dissociated whereas inorganic acids are strong acids that only reduce pH and cannot enter the cell. With their higher pKa (pH at which OA is half dissociated) value ranging between 3-5, OAs are partly dissociated and exert more effective antimicrobial activity. These undissociated molecules are lipophilic and pass more easily across the cell membrane into the microorganism. Once inside the microbial cell, the acid releases its proton (H+) in the more alkaline environment of the cytoplasm, resulting in a decrease of intracellular pH. This influences microbial metabolism, inhibiting the action of important microbial enzymes. The bacterial cell is forced to expend energy to expel the protons, leading to an intracellular accumulation of acid anions, depending on the pH gradient across the membrane. The anions within the microbial cell are thought to disrupt the metabolic processes in the cell, including RNA and DNA synthesis. This therefore affects cell multiplication and limits growth (Van Immerseel et al., 2006; FAFFENA, 2014). Improved gut structure of chicken is associated with reduced number of pathogenic intestinal bacteria (Dibner and Buttin, 2002). It has also been reported that organic acids are associated with Histo-morphometry of the gut by increasing the height of villus (Adil et al., 2011).

Despite many research studies have been carried out concerning the effects of various organic acids on gut health and integrity none of them are related to benzoic acid supplementation into broiler diets under commercial conditions. Therefore, the aim of the present research study is to investigate the responds to dietary supplemental protected benzoic acid with regard to antibacterial activity (inhibition of *E.coli* and *Salmonella* colonization), litter quality, pododermatitis and zootechnical performance parameters of broilers grown under commercial field conditions.

Material and Methods

A field trial was carried out in order to assess the impact of protected benzoic acid on gut microbiota, pododermatitis, litter quality and bird live performance parameters by replacement of tributyrin which is used as a standard OA only in starter diets of the broiler integration. In the present field trial benzoic acid was added for full cycle program into the standard broiler starter grower and finisher diets.

Housing

The field trial was carried out in a large broiler integration company located in northwest part of Turkey comprising breeder farms, hatchery, feed mill and broiler production houses operated by contracted farmers. In the Control Group 31.040 day-old unsexed Cobb 500 broiler chicks were allocated to one of commercial broiler house whereas in the treatment group 29.120 unsexed day-old chicks of the same Cobb 500 breed were placed into similar commercial house in the same commercial broiler production farm. Birds confined into two neighbour separate houses received the same practices with regard to management, health care and feeding, watering, heating, ventilation, vaccination and anticoccidial program. Overall broiler production practices were in line with Turkish and EU Animal Welfare Directives.

Dietary treatments

Dietary treatments consisted of tributyrin in the Control (the integrated broiler production company's usual organic acid administration program) and benzoic acid in the treatment group and both were supplemented into standard diets consisted of corn, soybean meal and full fat soybean. Field trial was terminated according to the company's slaughter program at 38d of age for the treatment group while for the control group birds were kept until 44d of age for slaughter.

Experimental diets

Control Group: standard feed + 750g/t coated tributyrin in starter feed (only for 10 days of age as usual program of the company) followed by standard grower, finisher diet, and withdrawal until end of trial.
Treatment Group: supplemented with protected benzoic acid (AVIMATRIX®, supplied by Novus Int.) at 500 g/ton of feed (equals ±250 of protected benzoic acid per ton of feed) to starter, grower, finisher and withdrawal diets until end of the trial.

Isolation of *Salmonella spp. and E. Coli* Colonization

A sufficient amount of birds (30 broilers/house) were randomly selected and killed by cervical dislocation at day 21st and at termination of the trial at 38d of age. Intestinal content sampling for quantitative measurement of bacterial load were done by cloacal swap for *Salmonella spp.* and for *E. Coli* count swap from Meckel's diverticulum region where *E. coli* density is presumed to be higher. All collected samples were adequately numbered based on the houses they were taken, and delivered (in ice boxes) to Bacteriology Department of Ankara University, Veterinary Faculty for bacteriologic analysis.

Foot Pad Lesion Scoring (Total FPD)

Foot-pad lesions were scored according to Swedish Method (Jong et al., 2012) by collecting broilers from the 2 flock at 21d and at 38d of age. 200 birds were sampled randomly from each of the broiler house along the diagonal axes. Foot-pad lesion necropsy and Total FPD were evaluated according to the Swedish Scoring System developed mainly for broilers and three FPD scoring categories were identified based on Swedish method as has generally been accepted within the EU countries:

Score 0: No lesions, slight discoloration on a limited area, mild thickening of the skin.
Score 1: Mild lesion; discoloration of the foot pad, superficial lesions, dark papillae.
Score 2: Severe lesion; ulcers or scabs, signs of haemorrhages or swollen tissue.
Final evaluation of FPS by flock was made as of the following equation:
Total FPD Score = (Number of birds with Score 1 x 0.5) + (Number birds with Score 2 x 2) *100/ Number of birds evaluated.

Litter quality

At 38d of age 10 litter samples were taken from each house by inserting a metal cylindrical cap (approximately 250 cc) towards the floor. Sampling was done longitudinally of the houses so as to avoid taking samples from nearby to nipple drinkers. Litter samples were put into nylon bags, they were numbered according to treatments and then sent to the same Bacteriology lab (of Veterinary Faculty) for water content determination.

Life performance parameters

Live weight (W) at start, and average daily gain (ADG) weekly and at the end of the trial, total feed intake (FI), FCR for each group and % mortality were determined and pursued on a weekly basis by mans of the farm electronic monitoring and computer system.

Statistical Analysis

The present field trial was set up according to Randomized Complete Block Design and the mathematical model was: Yijk= X + Ai + Bij + eijk; where: Yijk: was observation of the related parameter; X: general mean; Ai: the applied treatment effect; Bij: the block effect (broiler houses and environment); eijk: experimental error. The collected experimental data from the Control and Treatment groups were subjected to ANOVA according to SAS Software and comparisons of experimental group means analysed according to LSD or t-test. Statistical analysis of FPD scoring data was made according to Mann-Whitney U Test and Kruskal-Wallis Test (McDonald, 2014), since the characteristic of Foot-pad scores were qualitative and non-parametric abrupt distribution.

Results and Discussion

Live performance data indicated that broilers received protected benzoic acid grew better that the counterpart in the control group. Average daily gain (ADG) was 62.3 g/b/d in the control group, whereas in the benzoic acid received group was 62.8 g/b/d. It should be taken into account that despite the control group was held for longer period up to 44d of age vs. 39d of age, the treatment group received benzoic acid outperformed much better. Similar respond was detected in FCR too. Total feed consumption for the two groups were 141.440 vs 100.560 kg for the whole growth period and the average feed intake including weight of the dead birds were 4.56 and 3.45 kg/b respectively for control and treatment groups. Although average final weight of the Control and Treatment groups were respectively 2.743 kg (in 44 days), and 2.449 (in 39 days), related FCR values were 1.737 and 1.602. Therefore, to compare the two groups corrected FCR values were found and the calculated data indicated that in the benzoic acid group feed intake was less but growth rate was better and as a result corrected FCR was 1.692 with 4.5 points lower than the control group. Benzoic acid fed birds demonstrated a better feed efficiency in comparison with broilers receiving only butyrate source in their starter diets. Improvement in growth and feed efficiency by supplementation of benzoic acid fed broiler group could be attributed to increased gastric proteolysis and improved digestibility of protein and amino acid by (Kirchgessner and Roth, 1988).

Table 2. Effects of protected benzoic acid on broiler performance parameters

Performance parameters	Control	Protected Benzoic Acid
Number of birds housed	31040	29120
Age at slaughter (day)	44	39
Number of slaughtered broilers	29829	26645
Average Live Weight, g	2743	2449
ADG, g/bird/day	62.3	62.8
Total Feed Consumption, kg	141440	100560
Feed Intake, kg/bird	4.56	3.45
Corrected FCR*	1.737	1.692
EBI	345	359
Farmer Premium, TRY/bird	0.51	0.58

*For each 100 g difference in final weight 0.03 point is added

Likewise, when groups are compared in terms of European Broiler Index, EBI, (Average grams gained/day X % survival rate)/Feed Conversion X 10) where broiler's production results from different flocks grown in different places, still EBI of benzoic acid received group was 359 while in the tributyrin received group was 345. This outcome resulted in higher total profitability as seen by a higher EBI, and because of that, farmer premium in the benzoic acid received group was 0.58 TRY per broiler whereas in the control group was 0.51 0 TRY.

Table 3. Effects of benzoic acid on *E. Coli* colonization at 21 and 38 days of age

Treatments	N	Means	Standard Deviation	P value
At 21 days of age				
Control	30	$3,9 \times 10^5$ A	110667,38	0,001
Protected Benzoic Acid	30	$3,5 \times 10^5$ B	4548,58	
General	60	$3,7 \times 10^5$	6311,14	
At 38 days of age				
Control	30	$5,3 \times 10^4$A	454,85	0,0001
Protected Benzoic Acid	30	$1,5 \times 10^4$ B	454,85	
General	60	$3,4 \times 10^4$	2494,06	

A-B, means with different superscripts differ significantly (P<0,001)

Analysis of jejunal swap from Meckel's diverticulum at 21d of age has indicated that *E. Coli* colonization in the bird's gut received benzoic acid was significantly (P<0,001) lower than in the control group (3,5 x10^5 vs. 3,9 x10^5). Effects of benzoic acid on *E.Coli* colonization was more pronounced at 38d of age as the colonization log has reduced by 3 fold due to benzoic acid addition (1,5 x10^4 vs. 5,3 x10^4). Further reduction of *E.Coli* in the gut content is an obvious result of antibacterial effect of benzoic acid as the period of exposure to the protected organic acid was extended.

Table 4. Effects of dietary protected benzoic acid on *Salmonella* colonization at 21 and 38 days of age

Treatments	Date of sampling	Age	Salmonella spp. isolation
Control	16.02.2017	21-day	Positive
	03.03.2017	38-day	Positive
Protected benzoic acid	16.02.2017	21-day	Negative
	03.03.2017	38-day	Negative

Salmonella spp. isolation analysis results indicated that in both sampling at 21 and 38 days of age in the group received protected benzoic acid was negative while for the control group it was positive. The bacterial analysis indicated that protected benzoic acid is a potent antibacterial organic acid especially in the gut where pathogenic bacteria are receding. Similar effect of lowering pathogenic bacteria by benzoic acid has previously been reported Friedman et al. (2003). Positive effects such as improvement in growth, feed efficiency, gut health and broiler welfare obtained by protected benzoic acid used in the present study are in harmony with the results of previous studies where the findings generated explain very well the efficacy of benzoic acid for example; energy contribution to host animal (Jamroz et al., 2003); improvement in gastric proteolysis and digestibility of protein and amino acid in young broiler birds, reduction of ammonia production in the distal gut, thereby improved feed efficiency and growth performance of broiler birds (Kirchgessner and Roth, 1988); acting as energy source of the epithelia cells in the large intestine and terminal ileum and thereby improvement in the Villus height/Crypt depth ratio and positive effect on intestinal mucosa which helps in efficient feed absorption and assimilation (Roedigor, 1980; Chapman et al., 1995).

Table 5. Effects of dietary treatments on litter moisture content at 38 days of age

Treatments	Date of sampling	Age	Litter moisture %
Control	03.03.2017	38	41,0
Protected benzoic acid	03.03.2017	38	28,6

Data obtained from the present field study regarding to litter quality have indicated that significant reduction in the moisture level was observed in the group fed with the protected benzoic acid. Reduction of litter moisture level from 41.0% to 28.6% resulted in dryer litter was a not only an indication of healthier environment but also of better gut health lead to better growth and feed efficiency (Table 5). As a matter fact, improved litter quality by means of protected benzoic acid supplementation into the feed positively affected Foot-pad health in the group. Thus, Foot-pad lesions scoring results obtained by necropsy of 200 birds from each house related to control and benzoic acid groups have shown that in the first group prevalence of FPD scoring for (0), (1) and (2) grades were respectively 120, 75 and 5, whereas for protected benzoic acid fed group were 188, 11 and 1 respectively (Table 6).

Tablo 6. Analysis of FPD scores and Total FPD scores* of control and protected benzoic acid

Treatments	Number of observation	FPD Score prevalence		
		Score (0)	Score (1)	Score (2
Control [A]	200	120	75	5
Protected benzoic acid [B]	200	188	11	1

A-B, Treatments with different superscripts differ significantly (P<0,001)
*Total FPD Calculation: Control Group Total FPD= 0 x 120 + 1 x 75 + 2 x 5 = 85, Benzc
FPD= 0 x 188 + 1 x 11 + 2 x 2 = 15

Total FPD scoring results for protected benzoic acid group was significantly (P<0,0001) lower than in the control group (15 vs. 85). Meaning that benzoic acid supplementation positively affected gut health, prevented gut inflammation, diarrhoea and wet droppings. Therefore, litter condition was improved as was evident in the reduced moisture content of the litter. Overall the improvement in gut health by due to supplementation of protected benzoic acid resulted in healthier foot-pads. This is also important from bird's welfare which is also one of the essential reasons for farmer subsidy (as premium when Total FPD is lower than 80) in the western countries of EU like Holland and Germany.

Conclusion

The results of the present field trial have demonstrated that supplementation of protected benzoic acid into broiler standard diets at 500 g/ton dose level resulted in: negative *Salmonella spp*. isolation in caecum, significant suppression in *E.coli* colonization, reduction of Total Pododermatitis, increased average daily gain, improved FCR and improved European Broiler Index, and increased farmer premium.

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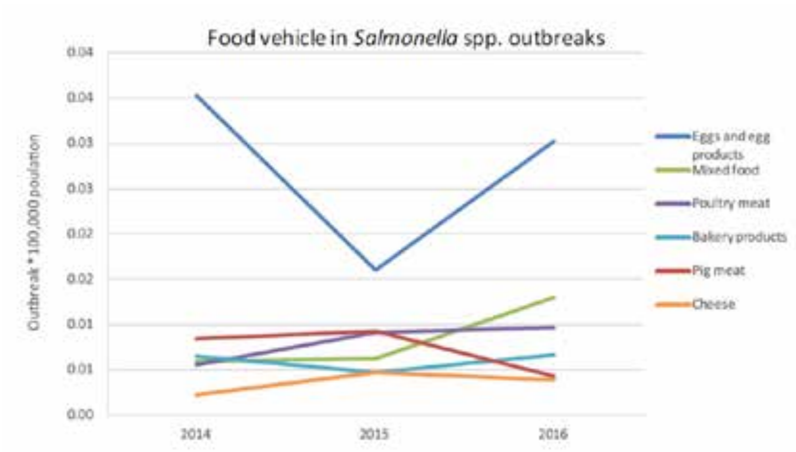
Satellite Symposia

SS[1] Microbiological Food Safety Risks in Poultry: What's New, What's True and What to Do?

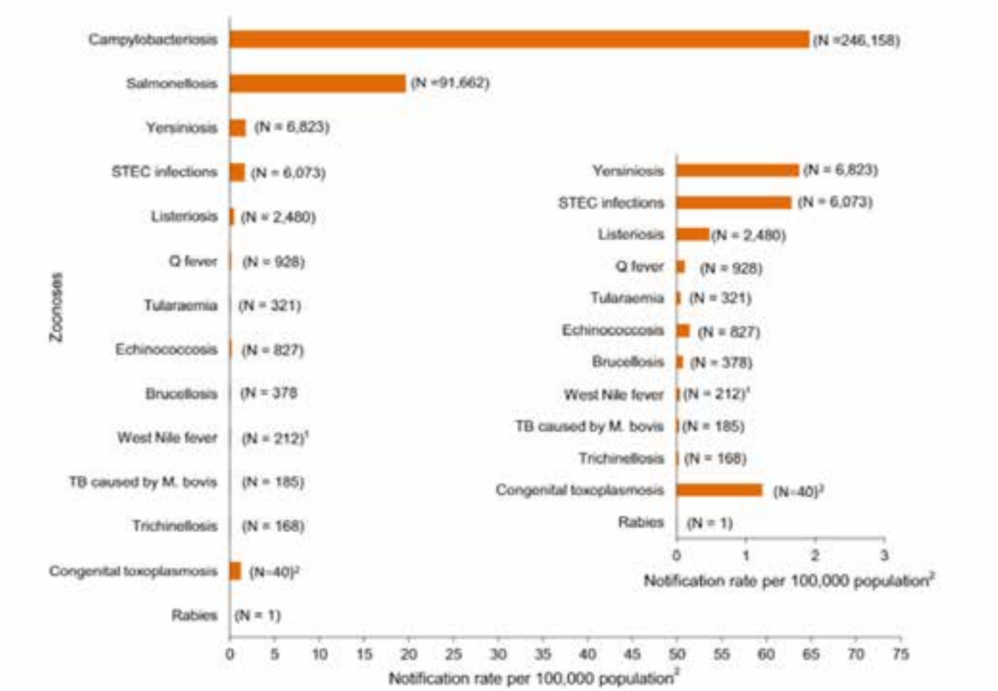
John Hanlin
ECOLAB, USA

Raw poultry processors play in reducing the risk of foodborne disease in poultry. Inspexx 210 - a biocide with peracteic acid and peroctanoic acid - reduces the risk of cross contamination of disease-causing bacteria from carcass to carcass from processing unit operations, process surfaces, knives and other cutting tools.

Protein demand will double by 2050 with population growth and changes in nutrition. In transitioning to food safety, foodborne disease accounts for approximately 600 million illnesses each year and about 420,000 deaths. Children account for almost one-third of deaths from foodborne disease. Some of the statistics of the World Health Organization and the European Food Safety Authority on the foodborne disease burden caused by poultry meat and Campylobacter and Salmonella, the two main bacteria causing disease in poultry, are as follows.



Outbreaks of food-borne Salmonella spp



The most commonly reported zoonotic campylobacteriosis in 2017

190,000 cases of campylobacteriosis are reported in the EU each year but the actual number of Campylobater infections in the EU may be closer to nine million annually. One statistic that caught the attention of the delegates was the infectious dose for Campylobacter may be as low as 500 cells, yet one study in the United Kingdom showed that 3.7% of carcasses may have more than 1,000 Campylobacter cells per gram of poultry meat. Another fact that poultry carcasses have been known to carry as many as 100,000 Campylobacter cells. He also discussed the disease burden attributed to Salmonella.

It is very difficult to control the risk of contamination of carcass meats during processing of raw meats and cross-contamination from equipment used to process meats such as knives and other cutting tools. Bacteria multiply on equipment used for processing meats. While EU regulations require the use of water of 82 ° C or an alternative system of equivalent effect, it is necessary to expose these meats to water of 82 ° C for more than 10 seconds to reduce the microbiological population of carcass meats by 90%. However, this is an impractical period in most poultry processing plants today. In contrast, exposure of poultry carcass meat to Inspexx 210 for one second produces the same level of microbiological inactivation as exposure to 82 ° C of water for 10 seconds. In addition, the Inspexx 210 biocide has the potential to neutralize bacteria and extend the shelf life of fresh poultry meat.

SS² Not All Probiotics Are The Same: Efficacy Of Enviva® PRO In Commercial Farms

Julio Villatoro
DuPont Nutrition & Biosciences, Holland

Unpredictable pathogens are on the rise due to the reduction or elimination of antibiotics in poultry production. Daily exposure to pathogens such as Escherichia coli, Clostridium perfringens and Salmonella can lead to poor flock performance and liveability. The energy that birds could direct toward growth must now be used to maintain health and overcome these challenges.

A healthy and mature gut can help keep these pathogens from wreaking havoc on a bird's health and performance. Today's accelerated live production schedule leaves little time for microbiota development—increasing the risk of reductions in body weight gain, and rising feed conversion and mortality rates. Natural mechanisms to fortify the gut exist and have powerful results.

Feeding probiotics from day one helps to quickly establish a positive microbiota and guards against colonization by coliforms—enhancing both production and animal welfare. But not all probiotic strains have the same mode of action, work effectively against the same challenges, or defend birds in the same way.

This is why it is beneficial to use a similarly diverse probiotic, with different strains. The three-strain Bacillus probiotic, Enviva® PRO, strengthens the gut structure, slows the growth of diverse non-beneficial bacteria and encourages the growth of beneficial bacteria, contributing to a healthier gut from day one.

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SS³ SYNCRA®-nizing Nutrition and Gut Health for Best Poultry Performance

Bart Hillen
DuPont Nutrition & Biosciences

In the context of reducing antibiotic use while maintaining high performance in broilers, both the animal nutrition and pharmaceutical industry realizes that animal health and nutrition should be much more integrated than realized before. Dupont Animal Nutrition identifies the three major pillars that define the physiological state of a broiler as nutrition, gut function and microbiome. All these features are in a fragile balance and all three should be taken into account when making decisions on how to improve animal performance. We also call this a "holistic" approach.

We will demonstrate an example of such a holistic approach is combining a health solution with a nutritional solution. What we see is that such combination can result in even better performance than would be expected from the solutions individually.

Enzymes are generally used to increase the nutritional value of a diet by enhancing the digestibility of specific raw materials. This can result in a boost in performance or it gives the option to use less digestible (and cheaper) raw materials and thereby decrease the costs of meat production. On the other hand, health solution come in a wide range and they have in common that they try to stimulate the immune function and save the animal spending energy on their immune response. One group of health solutions is probiotics, mostly aimed in modifying the gut microbiota composition in favor of beneficial bacteria and suppressing potential pathogenic bacteria. By doing so, we understand that this modification itself already enhances the gut structure, thereby having a positive impact on digestion. A healthy gut better absorbs nutrients and needs less energy spend on repairing inflammations and other immune related problems. So, a health solution in the first place turns out to have an attribution to digestion of the feed. This is confirming the overlap between the three pillars mentioned earlier.

However, we have discovered that it goes further than that. We observed that using a nutritional solution (enzymes) and a probiotic (gut health) actually amplifies the positive effects. Apparently the two solutions work better together than individually. This can be explained when we break down the modes of action. Enzymes, and carbohydrases in particular, break down substrates into elements that can be used by commensal bacteria in the gut. Especially bacteria that positively attribute to gut health thrive on these elements. Some of these bacteria have the capacity of producing short chain fatty acids (like propionic acid, butyric acid) that are generally known for their positive effect on gut health. When a carbohydrase is combined with a probiotic, the bacteria provided by the probiotic have a better chance of survival and thereby suppressing the growth and development of pathogenic bacteria even more. This can be measured in the hind gut. The abundance of several beneficial bacteria like bifidobacter, lactobacillus, eubacteria, Propionibacterium and commensal bacteria is greater when probiotic and enzymes are used individually. However, using them together results in even more of their bacteria. The two solutions are clearly amplifying each other.
The final aim remains the same: increase the digestibility of the feed and at the same time save the bird spending energy on their immune response. That energy should be diverted to growth, so essentially, we change the calorie conversion (kcal/kg body weight) and thereby decrease production costs.

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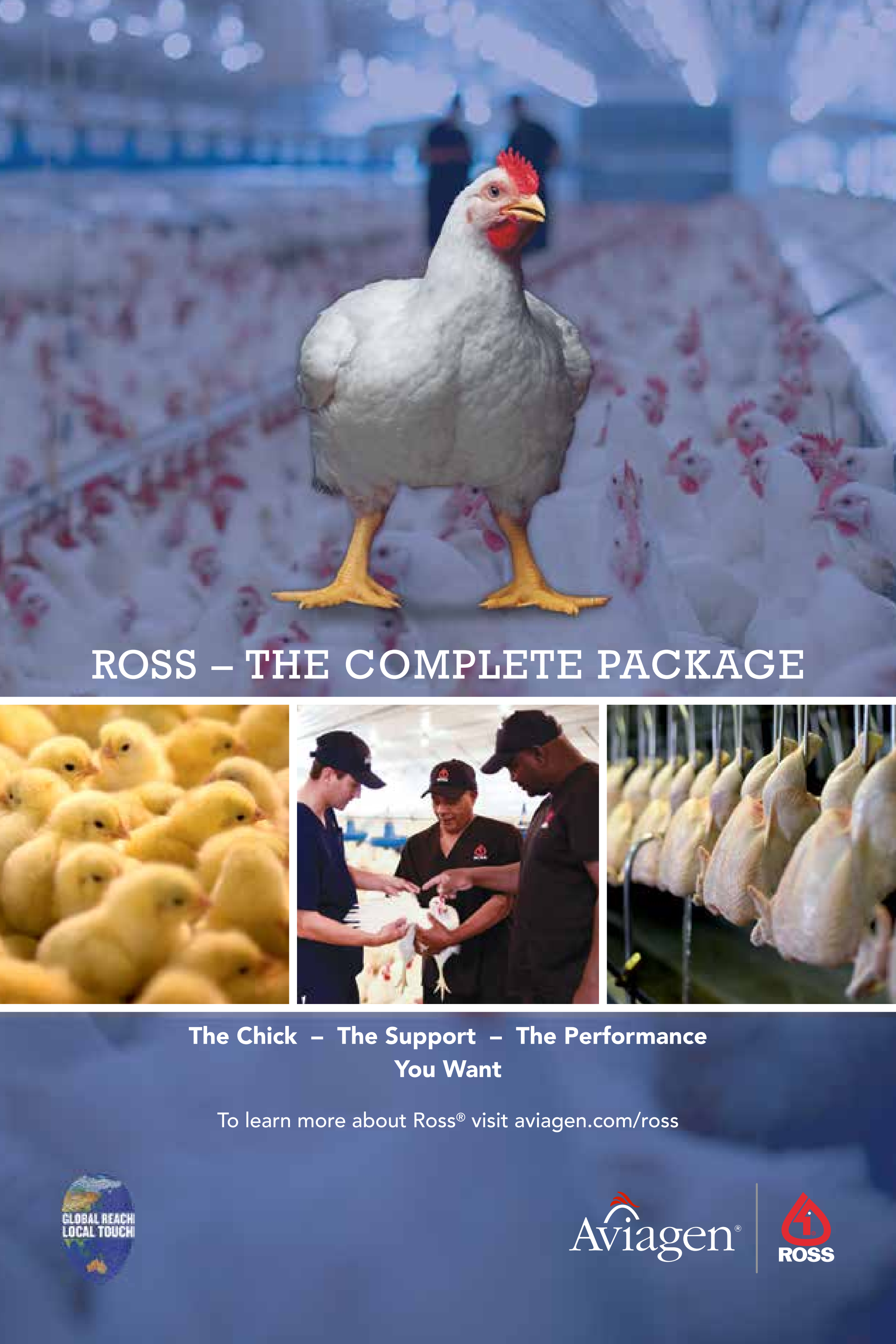
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