



# 3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS 22-26 April 2015 Starlight Convention Center &

22-26 April 2015 Starlight Convention Center & Sunrise Park Resort ANTALYA-TURKEY

# 3. ULUSLARARASI BEYAZ ET KONGRESI 22-26 Nisan 2015 Starlight Convention Center &

Sunrise Park Resort ANTALYA-TÜRKİYE

# PROCEEDINGS KONGRE KİTABI

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

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. 30 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.



# **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".







# 3rd INTERNATIONAL POULTRY MEAT CONGRESS

# PROCEEDINGS

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# Welcome

# **Organisation and Committees**

# **Program & Contents**

Oral Presentations	1
Poster Presentations	184



#### Dear Stakeholders of Poultry Meat Industry, Dear Friends

First two 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 and 2013 spring in Antalya with increased participation and success. The quality of the second congress has successfully reached to the international standards with it's high quality content including 65 oral presentation in 22 scientific session distributed into 3 different meeting room and 65 poster presentations. We have hosted total 1400 participants including family members and, approximately 900 active national and international participants in the second congress which has significant mission for our industry to extend and share the science and technology, and also strong integration to the world.

Turkish poultry meat industry grew up around 75% in a very short period, from 1.1 million tons poultry meat in 2007 to 1.9 million tons in year 2013. This huge growth made an incredible increase in our export by 665%, from 52 thousand tons to about 400 thousand tons for that short period. 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.

The dynamic power and most important capital behind the poultry industry are the science and technology. The main difference which isolates the poultry industry from backyard poultry farming is using research base science and technology over 100 years. As all we know very well modern or conventional poultry meat production is raising broiler or turkey chickens obtained entirely conventional (natural) breeding methods in controlled conditions which supply the most suitable environment that broiler needs and feeding them by considering science based knowledge to meet all nutrient requirements.

As a representative of Turkish Poultry Industry which has become the world's 8<sup>th</sup> 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 2015. 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 3<sup>rd</sup> congress will cover new devolopments, challanges, strategies for future considerations, safe poultry meat , sustainability besides importance of poultry meat for human nutrition and supplying enough and healthy meat for consumers.

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

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

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 3rd International poultry Meat Congress which will be held in beautiful Antalya from 22 April to 26 April 2015 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 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



## **Organisation and Committees**

### Chair of the Congress

**Prof. Dr. Necmettin CEYLAN** Ankara University

### **Organising** Committee

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### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

	<b>CONTENTS OF 3<sup>rd</sup> INTERNATIONAL POULTRY</b>	
	MEAT CONGRESS PROCEEDINGS	
NO	ORAL PRESENTATIONS	PAGE
	(Title. and Authors)	
O01	Contribution of Genetic Selection and Breeding Structures to the Economic	1
	Development of the Poultry Industry	
	Paul M. Hocking	
O02	Effects of Incubation Temperature on Hatchability and Chicken Quality	11
	Henry van den Brand and Bas Kemp	
O03	Effect of Using Cardboard or Plastic Viols on Hatchability	21
	Rana Dişa, Mehmet Bülüç, <u>Nezih Okur</u> , Serdar Özlü, Okan Elibol	
O04	Comparison of Some Production Characteristics of the White and Bronze	22
	Turkeys (Meleagris gallopavo) Under Intensive and Semi-Intensive	
	Conditions, I. Growth Performance	
	<u>Orhan Ozbey</u> , Hatice Ozer	
005	EU Regulatory Environment for Feed Additives Involving GMOs	23
	Ludovic Arnaud	
006	Amino Acids Ensure Sustainable Feed and Livestock Production	25
007	Michael Binder, Ogut Kose, Viktoria Scherer and Thomas Kaufmann	20
007	Challenges of Animal By Products in Turkey and EU Perspective	30
000	Nizamettin Şenkoyiu	24
008	Nuammer Cöngüoğlu	54
009	Effect of Cooking Methods (Gas and Electric Oven) and Times on	35
	Heterocyclic Aromatic Amines (HAAs) Formation in Chicken Döner	55
	Nesrin Özsarac, Nuray Kolsarici, Güliz Haskaraca, Eda Demirok Soncu	
010	Broiler Chicken Pectoral Myopathies	36
	Sacit F. Bilgili	
011	Ventilation Basics For Modern Broiler Housing	40
	James Donald	
012	Epigenetic Adaptation of Broilers to Inside Temperature of Broiler House	51
	Servet Yalçın	
013	Effect of Flock Age (27 Wk: Young; 37 Wk: Prime) Hatch Time and Litter	52
	Temperature on Broiler Performance	
	<u>Reza Shiranjang,</u> Serdar Özlü, Okan Elibol	
014	Ribotyping as a Tool for Epidemiological Typing of Salmonella Infantis in	53
	Broiler Production	
	Ozlem Şahan, K.Serdar Diker, Mehmet Akan	
015	Quality Control in Vaccine Production and EU Regulations	54
016		(7
016	Recent Developments in GMOs and Challenges in Turkey	67
017	Energy Transformation of Organia Wasta Matarial (Daultry Manusc) to	60
	Different Suitable Energy Generation Technologies	08
	Reinhard Enzenehner	



018	Challenges and Opportunities in Evaluation of Poultry Litter as Fertilizer	88
	<u>Mustafa Kaplan</u> Ahmet Şafak Maltaş	
019	Effects of Fresh and Composted Poultry Litter on Soil Aggregations	89
	Gökhan Çaycı, <u>Cağla Temiz</u> , Sonay Sözüdoğru Ok	
O20	Presence and Biocontrol of Listeria Monocytogenes in Turkey Meat	90
	Naim Deniz Ayaz	
O21	Class 1 Integrons in Multiple Antibiotic Resistant Salmonella Infantis Strains	91
	İnci Başak Kaya, Özlem Şahan, Mehmet Akan, Kadir Serdar Diker	
022	Human Salmonellosis Attributable to Poultry: Disconnect between Science,	92
	Policy, Regulation, and the Public's Expectations	
	Randall S. Singer	
023	Future Perspective of Poultry Nutrition	95
	Pınar Saçaklı	
024	Effects of Prebiotics, Probiotics and Essential Oils in Helping to Support	96
	Anticoccidial Activity	
	Mehmet Bozkurt	
O25	Effects of Herbal Vitamin D <sub>3</sub> Supplemantation and Calcium, Phosphorus	97
	Level on Growth Performance and Bone Development of Broilers	
	Necmettin Ceylan, <u>İsmail Yavaş</u> , A. Anıl Çenesiz, Nejla Kahraman, Semra	
	Güder	
O26	Strategies to Save Methionine in Broiler Diets	105
	<u>Cevdet Gökhan Tüzün</u> , İbrahim Çiftci	
O27	Effect of Relative Humidity During Incubation (0-18.5 D) on Hatchability and	106
	Broiler Performance	
	<u>Rana Dişa,</u> Uğur Can, Serdar Özlü, Nejla Kahraman, İsmail Ertonga,	
	Okan Elibol	
O28	New Aspects and Changes in Broiler Breeder Nutrition to Improve	107
	Performance and Hatchability	
	Eija Helander	
029	Enterococcus Spp. From Turkey Retail Meat Samples Resistant to	115
	Vancomycin and Their Antibiotic Resistance Profiles	
	Azam Azami Mahalleh, <u>Muammer Göncüoğlu</u>	
O30	Recent Developments in the Use of Exogenous Enzymes for Broilers	116
	Aaron .J. Cowieson	
031	Nitrogen Corrected Metabolizable Energy Value of Camelina Meal for 4-wk	122
	old Broiler Chickens	
	Ahmet Yavuz Pekel, and Olayiwola (Layi) Adeola	
032	Effect of Digestible Valine and Leucine Level of Broiler Grower Diet Based	123
	on Corn and Soybean Meal on Performance and Intestinal Development	
	Shahram Golzar Adabi, Necmettin Ceylan, Ibrahim Çiftci,	100
033	The Effect of Different Levels of Lysine on Performance and Serum	130
	Chemistry in Sexed Broilers	
	Abdur Rahman Sial, Rana Muhammad Atif, Anjum Khalique,	
	Muhammad Nasir, Muhammad Akram and Sami Ullah	100
034	Effects of Genetic Selection on Meat Quality	138
1	Paul M. Hocking	

3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI

035	Myths, Distortions and Lies About Poultry Meat and Production Systems	145
	Necmettin Ceylan	
O36	The False Perceptions About Chicken Slaughtering by Consumers and Media	146
	in Turkey	
	Musa Sarıca, Umut Sami Yamak	
037	Developments and Future Trends in Poultry Meat Processing	147
	Shai Barbut	
O38	Pastırma Flavored Chips Production From Chicken Meat	153
	Emine Çarkçıoğlu, Ramazan Efecioğlu, Burçin Taştan, Arzu Sarukan,	
	Yeşim Aktepe, Kezban Candoğan	
O39	Incidence of <i>C. perfringens</i> in Chicken Neck Samples Taken From	154
	Slaughterhouse, Molecular Characterization and Definition of It's Antibiotic	
	Resistance Profiles	
	<u>Güzin İplikçioğlu Çil</u> , Fatma Seda Bilir Ormancı	
O40	Effects of Rosemary Oil on Chemical and Microbiologycal Features of	155
	Chicken Meatball	
	<u>Özlem Pelin Can</u> , Sema Ağaoğlu, Süleyman Alemdar	
041	New Strategies to Improve Broiler Welfare	156
	Andy Butterworth	
042	Effects of Slatted Floor Housing on Animal Welfare in Broiler Production	161
	<u>Metin Petek,</u> Enver Çavuşoğlu, Ersin Topal, Cihan Ünal, İbrahima	
	Mahamane Abdourhamane	
043	Effect of Maternal Stress on Relative Asymmetry and Fear Behaviour of	162
	Broiles Reared Under Harsh Environmental Conditions	
	<u>Elif Babacanoğlu</u> , Servet Yalçın	
044	Poultry Investment Report for East Part(Malatya, Elazığ, Bingöl, Tunceli) of	163
	Turkey	
	Lokman Pirim, <u>Kamil Abdullah Eşidir</u>	
045	Further Understanding IBV is Essential to Effectively Control Respiratory Disease	164
	in Chickens	
	Haroldo Toro	
046	Intestinal Microbiome of Broilers and It's Interaction with Health and	167
	Disease Status of the Host	
	<u>K. Serdar Diker</u> , İnci Başak Kaya	
O47	Rapid Detection of Infectious Bronchitis Virus (AvCoV-IBV) by TaqMan Real	168
	Time RT-PCR and Phylogenetic Analysis of S1 Gene Variation	
	Yılmaz, H., Altan E., Çizmecigil, U., Gürel, A., Öztürk G., Erdoğan Ö.,	
	Çetinkaya B., Turan, N.	
O48	Effects of Heat Stress on Production Performance, Intestinal Integrity and	169
	Meat Quality in Broilers and Potential Nutritional Interventions	
	<u>Jan Dirk van der Klis</u> and Ester Vinyeta	
049	Effect of in ovo and Post Hatch Synbiotic Administration on Broiler Performance,	174
	Intestinal Histomorphology and Microflora from 0 to 21 Days of Age	
	Ali Calık, Ahmet Ceylan, Burcu Ekim, Shahram Golzar Adabı-i, Furkan	
	Dilber, Aley Gurol Bayraktaroglu, Turgay Tekınay, Pinar Sacaklı,	
	Doğukan Özen	



No	POSTER PRESENTATIONS	PAGE
	(Title, and Authors)	
P01	The Effects of Preslaughter Shackling on Some Stress Parameters, Fear, and	185
	Behavioural Traits in Broilers	
	Evrim Dereli Fidan, Mehmet Kenan Türkyılmaz, Ahmet Nazlıgül, Serap	
	Ünübol Aypak, Solmaz Karaarslan	
P02	Effects of Early Feed Restriction on Some Performance and Reproductive	186
	Parameters in Japanese Quail (Coturnix coturnix japonica)	
	<u>Evrim Dereli Fidan</u> , Mehmet Kaya	
P03	Nutritive Value of Camelina Meal and Its Effects on Performance and Meat	187
	Quality in Broilers	
	Ozge Sizmaz, Oguz Berk Gunturkun, Jürgen Zentek	
P04	Usage of Herbal (Solanum glaucophyllum) 1.25-dihydroxycholecalciferol	192
	(1.25(OH)2D3) in Broiler Breeder Diets	
	Gülay Deniz, Sümer Songur	
P05	Effect of Eugenol and Sauce on Broiler Baguettes Stored at +4 °C Contaminated	193
	with Salmonella spp.	
	Işıl Aydın, <u>Halil Yalçın</u> , Ali Arslan	
P06	Decontamination of Poultry Carcass with Chemical Methods	194
	Halil Yalçın, Işıl Aydın, Pınar Karatepe	
P07	Evaluation of Single Application of Cetylpyridinium Chloride (CPC) and	195
	Tri-Sodium Phosphate (TSF) on Broiler Carcass Contaminated with L.	
	Monocytogenes	
	<u>Halil Yalçın</u> , Ali Arslan	
P08	Insects as a Alternative Feed Sources	196
	<u>Yavuz Meral</u> , Ozgür Selçuk	
P09	Effects of Chitosanoligosaccharide Supplementation to Broiler Ratio on	197
	Growth Performance, Carcass Traits and Visceral Organs Weight	
	<u>Tuncay Tufan</u> , Cavit Arslan	
P10	Evaluation of Feed Grade Enzymes by Using <i>In Vitro</i> Digestive Tract Model	198
	<u>Sems Yonsel</u> , Mehmet Batum	
P11	Effects of Carrot Fiber and Carrageenan Scale on Chemical, Technological	199
	and Textural Properties of Chicken Hamburgers	
	Rabia Arslan, Ummü Kübra Ata, Omer Zorba, <u>Nursel Söylemez</u>	• • • •
P12	Effects of Dietary Olive Leaf Supplemention to Broiler Diets on Performance,	200
	Some Blood Parameters and Intestinal Microflora	
	Ismail Yavaş, Hatice Basmacıoğlu Malayoğlu	• • • •
P13	Chicken Meat Consumption Trends of Ege University Students	201
	Mert Saglam, Aslı Kıvırdık, Ebru Mencük	
P14	Factors Attecting Performance of Broilers in Integrated Broiler Operation	202
D1-	Nazlı Şelale Yiğiter, Servet Yalçın	
P15	Evaluation of Health, Economic and Performance Status Of Feed Additives	203
	Replacing The Antibiotic Growth Promoters After The Ban in EU and Turkey	
	<u>Emrecan Ozeler,</u> Arzu Gökdai	

3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI

P16	Effects of Breeding Age and Energy Restriction on Fattening Performance and	204
	Plasma Leptin Levels in Broilers	
	<u>Özlem Varol Avcılar</u> , Esin Ebru Onbaşılar	
P17	Hygine Application and It's Evaluation In Broiler Production	205
	Özlem Varol Avcılar	
P18	Foot Pad Dermatitis in Broilers And Turkeys	206
	Özlem Durna, Gültekin Yıldız	
P19	Does the Way for Exporting Poultry Meat to EU Make Zoning and	207
	Compartmentalization to Overcome the Challenges ?	
	Şebnem Gürbüz	
P20	Effects of Varying Dietary Valine Amino Acid Levels on Broiler Performance	208
	and Immune System	
	Murat Kaplan Gültekin Yıldız	
P21	Poultry Report for East Part(Malatya, Elazığ, Bingöl, Tunceli) of Turkey	209
	Lokman Pirim, Kamil Abdullah Esidir	
P22	Variation in Breast Meat Color and Its Relationship with Meat Quality in	210
	Broilers Slaughtered in Turkey	
	H.Cem Güler ve Servet Yalcın	
P23	Abdominal Fat Deposition in Broilers	211
	Sahin Cadırcı, Avfer Bozkurt Kiraz	
P24	Changes in Poultry Litter During Composting Period	212
	Gökhan Cavcı. Cağla Temiz. Sonay Sözüdoğru Ok. Gamze Denel	
P25	The effect of Sex on Some Welfare Indicators Measured Either on Farm or	213
	Processing Plants in Commercial Turkeys	
	Askın Güney, Mehdi Shekari Gündüz İlsever Sezen Özkan	
P26	Role of Poultry Nutrition in Preventing the Environment Pollution Related to	214
	Poultry Production	
	Saliha Bediz, Emre Sahin, Pinar Sacaklı	
P27	Evaluation of Poultry By Products as Protein Hydrolysates	215
	Avdın Erge, Ömer Zorba	210
P28	Economic Impact of Ectoparasites in Poultry Production Systems	216
120	Hatice Cicek, Hasan Cicek	
P29	The Impacts of National and International Standards on Organic Broiler	220
	Production in Developing Countries: Turkey Case	
	Kadir Erensov, Hakan Bayraktar, Ciğdem Seremet Tuğalay	
P30	Estimation of Ontimum Slaughter Age in Broiler Chicks	226
100	Hasan Cicek. Murat Tandoğan	
P31	High Hydrostatic Pressure: Innovations and Oppurtunities in Poultry Meat	227
	Industry for Inactivating Microorganisms	,
	Burak Bilen, Ahmet Yaman, Gülsün Akdemir Evrendilek	
P32	Effects of Different Supplemental Bentonite on Performance and Levels of	228
102	some Minerals in Blood Serum and Tissues in Broiler Rations	
	Hakan Üstüner. Seher Kücükersan	
P33	Effects of Selenium and It's Form on Broiler Breeder	229
1.00	Performance. Reproduction and Posthatch Chick Quality	/
	Özge Pamukcu, Necmettin Cevlan	
1		



P34	Consumer Views for Animal Welfare on Poultry Meat Consumption: Abant	230
	İzzet Baysal University Student Union	
	Canan Aslan, Ebru Cankurtaran, Eray Yalçınkaya, Esra Deniz, Özde	
	Beyazateş, <u>Ahmet Yaman</u>	
P35	Effects of Freezing and Frozen Storage on Poultry Meat Quality	231
	Eda Demirok Soncu, Nuray Kolsarıcı, Buğra Bilki, Feyza Yetimoğulları,	
	Hatice Üçler, Yeliz Bayar, Güliz Haskaraca	
P36	Recent Developments in Amino Acid Nutrition of Broiler Breeders	232
	H. Ozan Taşkesen	
P37	Effects of Beta Mannans as an Antinutritional Factors in Broilers and	233
	B-Mannanase Enzyme to Alleviate It	
	İsmail Yavaş, Gonca Sınacı, Necmettin Ceylan	
P38	Relationship Between Environment and Poultry Nutrition, and Developments	234
	İsmail Yavaş, Emine Yücesoy	
P39	Effects of A Phytogenic Feed Additive on Breast Meat Yield and Quality in Broiler	235
	Alban Llorca	
P40	Effects of Glyceryl Polyethylene Glycol Ricinoleate on Nutrient Utilization	237
	and Performance of Broiler Chickens	
	S.A. Kaczmare, M. Bochenek, A-C. Samuelsson, A. Rutkowski	
P41	Effect of Dietary Supplementation of Thyme Oil and Vitamin Combinations	242
	on PerformanceTraits in Heat Stressed Broilers	
	Savaş Sarıözkan, Berrin Kocaoğlu Güçlü, Yusuf Konca, <u>Eray Aktuğ,</u>	
	Mahmut Kaliber, Selma Büyükkılıç Beyzi	
P42	Investigation of Proteolytic and Lipolytic Spoilage Causing Bacteria for	243
	Selling Chicken Meat in Istanbul, Turkey	
	<u>Aysen Coban</u> , Ali Aydın, Gulay Merve Bayrakal, Ruveyda Gunaydın,	
	Mert Sudagıdan	
P43	Assessment of Poultry Sector in Turkey in Terms of Waste Generation and	244
	Waste Valorization	
	<u>Ebru Deniz</u> , Kezban Candoğan	
P44	The First Bivalent Salmonella Live Vaccine for Chicken, Turkeys and Ducks	245
	Windhorst, D., Wefstaedt, P., Hagen, S., Monsalve, F., Regenhard, P., and	
	I. Schroeder	
P45	Nutrigenomics; An Emerging Science in Poultry Production	249
	Hasan Hüseyin İpçak, Raziye İşık, Ahmet Alçiçek	
P46	Does Phytase Mean just Ca and P in Broiler Nutrition ?	250
	A. Anil Çenesiz	
P47	Bio-Efficacy of Feed Proteases In Poultry and Their Interaction with Other	251
	Feed Enzymes	
	L.F. Romero And P.W. Plumstead	
P48	The Dietary Supplemental Effect of Anticoccidial Drug Monensin and	259
	Oregano Essential Oil on Digestive Enzyme Activities, Intestinal Morphology	
	and Cecal Microbial Shedding of Broiler Chickens Challenged with Mixed	
	Eimeria Spp.	
	Gökhan Ege, Mehmet Bozkurt, Nuran Aysul, Ahmet Engin Tüzün, Ayşe	
	Ebru Borum, Erol Bintaş, Bahattin Koçer	

# **ORAL PRESENTATIONS**



#### O<sup>01</sup> Contribution of Genetic Selection and Breeding Structures to the Economic Development of the Poultry Industry

#### **Paul M. Hocking**

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#### Summary

The rapid increase in the supply of cheap cereals following the end of the second world war, accompanied by the adoption of technology for large scale housed production systems, including environmental controls and vaccination to control disease, was associated with the provision of specialised hybrids selected for rapid growth and increased feed efficiency. In a relatively short time chicken meat developed from a luxury product to became relatively cheap and affordable. Specialised international companies were established to supply various components of intensive broiler production systems. An essential component of these developments was genetic selection of lines of chickens that exploited the potential of these production systems. Breeding programmes were developed that utilised sophisticated statistical techniques and methods for measuring "new" phenotypes, including welfare and health related traits, such that by 2000 about 85% of the improvement in productivity could be attributable to genetic changes and only 15% to improved nutrition. The development of specialised male and female lines of broilers maximised genetic gains in the final broiler and crossbreeding exploited hybrid vigour for reproduction and fitness traits. Parallel development of efficient breeding systems for rapidly multiplying genetic improvement to commercial flocks was made possible by the exceptional reproductive potential of the chicken. The feed efficiency of modern broilers surpasses that of ruminants, pork and alternative sources of poultry meat. This in turn contributes to reducing the demand for arable land and the environmental impact of producing meat. The demand for broiler meat is predicted to more than double by 2050 due to the increasing size of the human population and by the increased demand associated with rising affluence in developing nations. Genetic selection of broiler chickens has therefore made by far the largest contribution to the economic development of the poultry meat industry. Furthermore it is likely that broiler meat will continue to increase at the expense of alternative sources of animal protein and make a substantial contribution to reducing the impact of meat production on the environment. Alongside innovations in management and technical advice, genetic selection in balanced breeding programmes will continue to make a substantial contribution to solve problems that arise in the future through the development of new traits, new methods for measuring old traits and the adoption of novel genetic techniques.

#### Introduction

The period from 1950 to 1975 saw the rapid development of farming in many parts of the world from labour intensive, mixed livestock and crop systems to increased specialisation, mechanisation and widespread use of artificial fertilisers, herbicides, pesticides and fungicides. Crop yields increased, cereals became cheaper and as a consequence it was became profitable to feed animals on high energy, high protein cereal sources. These developments were associated with the creation of organisations that circumvented traditional pedigree breeding structures and adopted objective breeding programmes based on scientific principles. Alongside the resulting

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

improvements in genetic potential, changes in housing design, vaccination and nutrition facilitated the development of sophisticated, large scale, efficient and economic systems of production for poultry. Genetic selection for broiler traits quickly led to the creation of two separate commercial enterprises for chicken eggs and poultry meat production. This was in sharp contrast to the previous dual purpose systems where chicken meat was a relatively expensive by-product of surplus males and culled hens from egg laying flocks. The period from 1975 onwards was associated with increasing size of both flocks and commercial enterprises and the consolidation of breeding organisations on an international scale. Similar processes occurred in ducks, turkeys and the minor poultry species (geese, quail and guinea fowl).

Currently there are estimated to be over 60 billion broiler chickens, 2.6 billion ducks and 0.6 billion turkeys worldwide (FAOSTAT, accessed 1/4/2015) meeting an ever increasing demand for poultry meat. It is estimated that the demand for broiler chickens will increase by 2.4% per year from 2011 to 2020 [1] and is likely to double by 2050. This will occur because expansion of demand for more meat is estimated to be +15% in developing countries, +69% in the least developed and +103% in developing economies that also contain the largest proportion of the human population [2]. As these economies develop, the demand for chicken will increase in the human population.

There are many advantages for poultry meat: it is cheap, tender, low in fat, high in unsaturated fatty acids, balanced *n*-6 to *n*-3 PUFA ratio, high linoleic acid, vitamins and minerals, low in sodium and cholesterol, and has a balanced amino acid profile. Furthermore the poultry meat industries have led the way in innovation by developing new markets through added value and further processing. Production of broiler chickens in particular does not require large areas of land and the technology can be imported rapidly via turnkey projects virtually anywhere in the world. It is confidently predicted to take over the number one spot as the major source of meat in the next decade if it has not already done so. What then are the current challenges facing the broiler meat industry?

#### **Current and Future Challenges to the Poultry Meat Industry**

The most important challenges for the poultry meat industry are related to its considerable success: these challenges are the result of the growth of the human population on the demand for poultry and the effects on climate change leading to increasingly scarce resources of land, energy and water, and the effect of these processes on the environment (Table 1). Added to these changes is the impact of legislation to protect animal welfare and the environment, and the appearance of new diseases and very virulent forms of old diseases. The emergence of new and niche markets provides both a challenge and an opportunity that can be seen in the expansion of Label Rouge poultry meat in France and other "quality" brands of poultry meat such as the seasonal Traditional Farm Fresh turkeys in the UK. However these developments will hardly feed the world and will likely remain a relatively small component of the market for poultry products in developed countries.



uuseu oy wond population growin [oused on 5].					
Resource	Increase to 2030	Increase to 2050	Impact on poultry		
People	15%	30%	Greater demand		
Land area	5%	Nil	Competition for feed		
Land quality	Decline?	Stable?	Competition for feed		
Energy	+45%	+100%	Higher feed costs		
Water	+35-60%	+100%	Change location		

**Table 1**. Potential impact on the production of poultry meat from competition for key resources caused by world population growth [based on 3].

Higher demands for meat will exacerbate the pressure on natural resources such as land, water and energy with consequential impacts on climate change and the environment. There may also be political instability caused by greater inequality and pressure on the poor in countries that fail to adapt, and through increased competition between countries for scarce resources such as water. Whereas this catalogue of negativity has huge implications for the global community, a switch from livestock to meat from poultry will contribute to ameliorating the effects of these changes by providing high quality food at high feed conversion efficiencies. On the other hand, poultry feed supplies will have to compete for land, water and fertilizer for crops that could be used directly to feed humans and contribute to climate change though the use of fossil fuel for fertilisers, crop protection and transport of feed.

The world is undoubtedly experiencing changes in the climate that are associated with an increase in greenhouse gases. Weather systems may become more variable and extreme in future, adding to the pressure on food production for the increasing human population. These factors will affect where food is grown and how much it costs but poultry production may not be greatly affected. Most industrial production takes place in confined spaces that are frequently environmentally controlled. Techniques for managing hot environmental temperatures already exist in many areas of the world and will continue to expand and adapt to new challenges. Furthermore production could move to areas with more benign climates at greater altitude or higher latitudes.

The production of meat from poultry also has a direct impact on the environment through the effects of nitrogen and phosphorus runoff on water quality, ammonia and nitrogen emissions into the air, and contamination of the soil. Indirect environmental impacts occur through the growing of crops, the impact this has on soil degradation and water pollution, and the manufacture and transport of feed, all of which are heavily dependent on fossil fuels that in turn contribute to climate change. However, in the last half of the 20<sup>th</sup> century, animal breeders made a substantial contribution to the long term sustainability of poultry meat production by selecting for lines of birds that are highly efficient at converting feed to food (Table 2). This trend will continue and make a substantial reduction on the environmental impact of broiler chicken meat production on the environment, in terms of land use, water utilisation, atmospheric emissions and of course feed, are all substantially lower compared with the other farm species [4].

Tuble 2. Summary of feed of	sinversion radios (r erc) for 7 meat pre	duction systems [5].
Species	Production	FCR
Broiler	36 d	1.69
Turkey	126 d	2.46
Geese	98 d	3.26
Ducks	42 d	2.41
Pigs	22 weeks	2.99
Sheep	75 kg	10.5
Beef	600 kg	10.4

Table 2. Summar	v of feed co	nversion ratios	(FCR)	for 7 me	at production	systems	[5]	١.
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#### Quantifying the Contribution of Genetic Selection of Broiler Chickens

#### Broiler traits

Body weights and carcass quality traits in 37 pure bred lines of broilers, layers and traditional breeds are presented n Table 2. Collectively these lines had a 42 day body weight ranging from 0.4 to 3.2 kg and even within the broiler lines there was a two-fold difference in body weight. Live weight, yields and muscle quality of the layer and traditional lines were similar whereas broilers had higher carcass and breast muscle yields. Breast muscle was lighter in colour, there were more haemorrhages on the surface of the muscle and plasma creatine kinase activity, a marker of muscle cell damage, was greater in broilers compared with layers and traditional lines. However, taste panel assessment of the texture, flavour and overall liking of cooked breast meat showed that broiler meat was preferred over that from the unselected birds.

Trait	Broiler	Layer	Traditional	SED
Production traits				
Live weight (ln g)	7.78 <sup>a</sup> (2387)	$6.30^{b}(544)$	6.37 <sup>b</sup> (586)	0.080***
Carcass weight	713 <sup>a</sup>	$608^{\mathrm{b}}$	614	8.6***
(g/kg)				
Breast (g/k)	291 <sup>a</sup>	175 <sup>b</sup>	182 <sup>b</sup>	8.0***
Muscle quality				
Creatine kinase (ln	6.69 (805)	5.33 (205)	5.35 (210)	0.078***
$IU L^{-1}$ )				
Lightness (L*)	55.9	53.0	52.0	0.668***
Haemorrhage score	3.74	2.04	2.08	0.180***
Taste panel scores				
Texture	6.03	5.90	5.46	0.201**
Chicken flavour	3.84	3.59	3.52	0.131*
Overall liking	4.28	3.97	3.79	0.127***

**Table 3.** Live weight and meat yields, muscle quality and taste panel assessments of for 37 genetic lines of chicken [6].

The role of genetic selection on feed conversion has been adequately demonstrated by the huge improvement in contemporary broilers compared with previous generations. Zuidhof et al. (2014) compared random bred genetic pools created in 1957 and 1978 and a commercial line of hybrid broilers from 2005. Average body weights at 56 days of age respectively were 0.9, 1.8 and 4.2 kg and feed conversion rates from hatch were 2.85, 2.14 and 1.92. Havenstein et al. (2003) also compared the 1957 random bred control with a line of broilers in 2001 that were fed on a contemporary 2001 diet or a diet formulated to represent diets fed in 1957 (Table 3). Body weight was about 5 fold higher, FCR was substantially lower and breast muscle yield was higher in the 2001 broilers compared with the historic line, consistent with the later report of Zeidhof et al. (2014).



Havenstein *et al.*'s research [7, 8] was particularly valuable as it was possible to determine the relative change from genetic selection to be compared with the improvements in nutrition. Genetic improvement was responsible for 87% of the change in body weight and 84% in feed conversion leaving nutrition responsible for about 15 % of the change in performance over a period approaching 50 years. Fleming and colleagues compared a 1972 broiler with the 2005 (Ross 308) broiler derived from the same ancestral lines as the 1972 bird. The estimated yearly change in live weight, FCR, mortality, carcass yield, breast yield and abdominal fat respectively were 44.1 g/d, -0.018,-0.026%, 0.082%, 0.192% and -0.052% and the authors concluded that improvements in production traits over this 33 year period had been obtained without compromising mortality or leg health.[9, 10]. Taken together these reports demonstrate the considerable progress made by genetic selection for FCR and carcass quality over a long period of sustained genetic selection. Furthermore, the next section will show that this change in broiler productivity has also been accompanied by improvements in reproduction.

1000000000000000000000000000000000000					
Trait	200	1 diet	1957 diet		
	1957	2001	1957	2001	
Body weight, g	578	2672	539	2126	
Feed conversion, g/g	2.14	1.63	2.34	1.92	
Breast, %	11.6	20.0	11.6	17.4	
Fat, %	10.9	13.7	8.5	12.2	
Mortality, %	2.4	3.6	1.8	3.6	

**Table 4**. Mean of male and female performance at 42 days of age of a random bred flock of broilers established in 1957 and a commercial hybrid from 2001 fed on a 1951 or 2001 diet [7, 8].

#### **Reproduction traits**

Direct comparison of some reproductive traits of random bred broilers from 1955 and a commercial hybrid in 2013 is presented in Table 5. The modern line laid heavier eggs with higher hatchability reflecting lower rates of infertility and embryo deaths whereas the percentage of culled chicks were not statistically different. Unfortunately Collins et al. [11] did not present estimates of the egg production of the heritage and modern lines. However, phenotypic improvements in reproductive traits estimated in commercial flocks of Ross 308 parent stock showed an annual improvement in hen housed egg and chick production, increased hatchability and decreased mortality (Table 6). Even though these traits have a low heritability substantial gains were recorded over the study period in all these traits during a time when there was little change in the production environment. Clearly the suggestion that reproduction has declined in modern broilers is not substantiated by these data: Taken together the reports show clearly that genetic improvement in reproductive traits in commercials broiler breeding has occurred and that this has been reflected in the commercial environments in which these birds are housed. However, during the rearing period, a substantial degree of feed restriction is necessary to achieve these rates of egg production (see below).

a modern broiler from 2013 [11].		
Trait	ACRB, 1955	Cobb, 2013
Egg weight (g)	50.0	63.2
Percent hatch	86.7	89.2
Infertile %	4.0	2.7
Early dead %	5.0	3.7
Late dead & pip %	4.0	2.6
Cull chicks %	0.1	1.4

 Table 5. Comparisons of egg weight and reproductive traits of a random bred line from 1955 and a modern broiler from 2013 [11].

**Table 6**. Annual trends in hen housed egg and chick production, hatchability and hen mortality to 60 weeks of age in commercial flocks of Ross 308 [12].

Trait	Male line	Female line	Parent stock
Total eggs	0.4	1.7	1.7
Total chicks	0.6	1.8	1.3
Hatchability	0.25	0.47	0.09
Hen mortality	-0.69	-0.05	-0.41

#### **Organisation of Poultry Breeding Structures**

There are relatively few breeding organisations producing parent broiler stock and just 3 international companies dominant the market [1]. A similar situation exists in the turkey and duck industries and in all three species there are smaller suppliers meeting largely niche markets.

Each species is served by a similar pyramid breeding structure with the pedigree lines at the apex followed by multiplier flocks that meet the needs of commercial producers for parent stocks. This structure has several important features: Several lines are maintained at the pedigree level and are crossed in different combinations to produce parents that are in turn mated to produce the commercial product; the final offspring may be the result of crossing three or four lines and different combinations of lines may be used for alternative markets; and the breeding pyramid results in maximising the number of offspring from the improved genetic potential of the pedigree population. Female parents are always first crosses to maximise heterosis for viability, egg and chick production; genetic selection of the male pedigree line is primarily for feed conversion, breast yield, fertility and welfare traits whereas the female lines are also selected for reproduction traits thus overcoming to some extent the negative correlation between egg production and growth . The reproductive potential of the broiler female that may produce 100 or more offspring and a male that will fertilise at least 10 hens results in very high selection intensities that can be spread over a large number of traits while still making significant genetic progress in the primary commercial traits of feed conversion and breast meat yield. However, an important consequence of the breeding structure is that there is a "multiplication lag" of 4 to 5 years for the results of successful genetic selection in pedigree lines to be observed in commercial broilers.

#### **Genetic Challenges for Broiler Chickens**

Improvement of gait and musculoskeletal disorders

Genetic correlations between walking related traits and body weight are low so that genetic progress can be made (Table 7). Indeed, it has been demonstrated that genetic improvement of FCR and breast yield has been associated with positive changes in skeletal disorders and walking ability following genetic selection in a balanced breeding programme over a 25 year period [13-15].



Trait	Heritability	Genetic correlation with body weight
Body weight	0.36	_
Leg defects	0.05	0.18
Crooked toes	0.05	0.14
Tibial dyschondroplasia	0.17	0.19
Foot pad dermatitis	0.24	-0.05
Hock burn	0.08	0.30
Gait score	0.28	0.29

Table 7. Heritability and genetic correlations	with body weight o	of traits associated	with walking
ability in broilers [13-15].			

#### Muscle and meat quality

White striping, wooden breast and variable breast meat colour have recently become significant concerns to the broiler industry affecting the incidence of carcass condemnations, processing quality and retail presentation. These aspects are covered in more detail elsewhere in these proceedings (Bilgili, pages XXX; Hocking, pages YYY). Substantial genetic variation for muscle and meat quality attributes exists [7]. Recent estimates in purebred commercial broiler lines have found low heritability for deep pectoral myopathy and wooden breast, moderate heritability for white stripping, and low to moderate genetic correlations with body weight and breast meat yield. This analysis showed that non genetic effects contributed to more than 65% of the variance in the incidence of breast muscle myopathies, hence while breeding could make a contribution in the long-term, it is also essential to understand the environmental factors that contribute to the field incidence.

#### Reproduction

The welfare of feed restricted boiler parent stock during rearing has been questioned on the basis that the degree of restriction is sufficient to cause chronic hunger. This issue has recently been assessed by the European Union [16] and will doubtless become more important in future, at least in developed nations. Broiler parent stock are feed restricted to improve liveability and feed use but the large reduction in body weight is absolutely necessary to maintain commercially viable rates of egg production and fertility (Table 8).

Trait	Ad libitum	Restricted
Body weight, kg	5.3	3.7
Mortality, %	46	4
Egg production, n	58	157
Hatch of eggs set, %	43	86
Feed intake g/d		
0-24 weeks	163	63
24-37 weeks	192	157
37-60 weeks	142	151

**Table 8**. Body weight, mortality, egg production, hatchability and feed intake of broiler breeder females fed *ad libitum* or feed restricted from hatch to 60 weeks of age [17].

Low rates of lay in *ad libitum*-fed broiler breeders are associated with multiple ovulation that interferes with the capture of ovulated yolks by the infundibulum (Table 8). This results in internal ovulations that are reabsorbed in the body cavity, or the production of defective egg shells (shell less, membranous, double yolked, slab sided, etc.) by the presence in the oviduct

of 2 or more yolks at the same time. Feed restriction to control body weight during rearing and in lay decreases the proportion of yellow follicles (future egg yolks) and leads both to increased egg production and longevity (Table 9). In a large field study, Alvarez and Hocking [18] showed that in feed restricted birds the incidence of multiple follicles in the first half of the laying period ranged from 10-30% of breeders that would in turn lead to a decrease in the rate of lay of settable eggs. Genetic selection to decrease the propensity for multiple ovulations would contribute to increased egg production and, in the longer term, allow flock owners to feed breeders to optimise their welfare without recourse to severe feed restriction. Interestingly, feed restriction is relatively ineffective in turkey hens but is used successfully in ducks, as discussed in more detail by Hocking [19]. Realistically, genetic selection for single ovulations is difficult and awaits the development of indirect DNA-based methods.

**Table 9**. Body weight, number of yellow follicles and the proportion of yellow follicles developing as pairs of similar size and ovulable status at the onset of lay in a line of broiler chickens [20].

Feeding	Body weight, kg	Yellow follicles, N	Multiple follicles, %
Ad libitum	5.6	11.8	64
Feed restricted	3.9	7.4	27

#### Inevitability of Genetic Challenges

Genetic improvement in broiler traits that contribute to profitability of the industry with continuous reduction of the negative impact on the environment, and adaptability to a range of environments in balanced breeding programmes, will continue into the future [21]. Intense natural and artificial selection results in selective sweeps [22] which increases the frequency of linked recessive genes (alleles) through linkage to selected genes [23] exposing cryptic genetic variation. Genetic selection results in linked (and unlinked) genes with a positive correlation reaching fixation, leaving recessive genes with negative correlations or those that are deleterious to become more frequent. Because selection is intense, and effective populations sizes are relatively low, these genes may become homozygous giving rise to negative correlations and unexpected correlated responses to selection. Hocking [24] argued, on the basis of genetic theory, that unexpected consequences of genetic selection, such as leg and muscle defects, were therefore inevitable and that genetic selection in a balanced breeding programme to reduce these effects are essential. To a large extent breeding companies have been successful in doing so over the last 20-30 years [13, 14]. In addition, genetic drift may increase the frequency of deleterious alleles and to mitigate this effect, breeders maintain large effective population sizes, minimize relationships between relatives and maintain several selected and unselected reserve lines [1]. Furthermore the negative correlations with broiler traits that do arise are generally of low magnitude (e.g. Table 6) and do not compromise genetic progress in the major production traits in balanced breeding programmes.

#### **General Conclusions**

Genetic selection combined with breeding structures that multiply genetic improvement into commercial flocks is responsible for 85% of the change in feed efficiency since the 1950s and has increased the yield of high value breast meat by about 2-fold during the same period. Poultry meat has made, and will continue to make, a substantial contribution to reducing the impact of meat production on the environment in the face of an increasing human population and rising demand for meat linked to increased affluence.



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#### O<sup>02</sup> Effects of Incubation Temperature on Hatchability and Chicken Quality

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#### Summary

Due to genetic selection, broiler chickens have been changed in the last decades, which has consequences not only for the rearing phase, but also for incubation. The most important incubation factor for obtaining optimal results is incubation temperature. Effects of relatively small changes in incubation temperature have been shown to have considerable effects on hatchability, chicken quality and later life performance. Additionally, recent studies indicated that incubation temperature appears to have effects on bone development and (intestinal) health of broiler chickens as well. In this paper consequences of incubation temperature on indicated aspects are summarized and a proposed mechanism is discussed.

#### Introduction

Poultry production has been changed strongly in the last 50 years. In broilers, body weight gain between day 36 and 42 of age was 20.6 g/d in 1957 line chickens, whereas this was increased to almost 100 g/d in 2005 line chickens (Zuidhof et al., 2014). At the same time, the FCR between day 0 and 42 of age decreased from 2.9 to 1.7. These results from genetic selection and improved management not only affect post-hatch performance of the broiler chicken, but also has consequences for the pre-hatching phase (incubation). In the same 50 years, the incubation process in commercial poultry industry has been changed dramatically from small sized stilldraught incubators to forced-draught incubators containing up to 100000 eggs per incubator. The forced-draught incubator was first used as a multi-stage machine, meaning that each incubator contained eggs of different ages. In the last decades, it is has been recognized that the multi-stage incubators do not completely fulfil the embryo requirements of the modern breeds, meaning that hatchling quality was not optimal (Hill, 2000). Therefore, single-stage incubation was introduced, in which only eggs of one age are incubated in an incubator. Because eggs in a singlestage incubator are all in the same developmental stage, machine settings, such as temperature, relative humidity and CO2 can be adjusted according to the changing embryonic requirements during the different phases of embryonic development (French, 1997; Hulet, 2007).

One of the most important environmental factors affecting embryonic development is incubation temperature. (Decuypere and Michels, 1992; Meijerhof, 2009). Incubation temperature is not only affecting embryonic development and in turn hatchling quality, it also might have long term effects on growth, performance and health in later life. The aim of this paper is to shortly review consequences of incubation temperature on embryonic development, hatchling quality and later life performance.

#### Embryo temperature

Although it has already been recognised for more than 50 years that embryo temperature would

be the most important variable to control during incubation (Romijn and Lokhorst, 1951), only in the last decade embryo temperature (or eggshell temperature (EST) as a reflection of embryo temperature) is used to control the incubation process in commercial hatcheries (Meijerhof and Van Beek, 1993; French, 1997). Historically, not the EST, but the air temperature of the incubator (machine temperature) is controlled and maintained between 36 and 38oC (Decuypere et al., 2001). However, when machine temperature is maintained throughout incubation at a fixed level of 36 to 38oC, embryo temperature will increase at later stages of incubation, due to the increase in embryonic heat production (see below). Studies in the last decade have shown that a constant EST of 37.5 to 38.0oC, rather than a constant machine temperature, results in the highest hatchability and chicken quality (Lourens et al., 2005, 2007; Joseph et al., 2006; Leksrisompong et al., 2007).

The EST is the result of embryonic heat production and heat transfer from the egg to the environment. Embryonic heat production is very low at the first week of incubation, but increases exponentially after day 7 to 9 of incubation (Lourens et al., 2007, Molenaar et al., 2010, Nangsuay et al., 2013). Between day 15 and 18 of incubation, heat production reaches a plateau phase of approximately 140 to 150mW/egg (Dietz et al., 1998; Lourens et al., 2006a, 2007; Molenaar et al., 2010; Nangsuay et al., 2013). After internal pipping at day 19 of incubation, embryos changes their respiration from the chorio-allantoic membrance (CAM) to lung ventilation, resulting in a strong increase in heat production at that stage (Rahn et al., 1981; Janke et al., 2004; Figure 1).



Figure 1. Embryonic heat production, evaporative heat loss, embryo temperature and machine temperature in relation to the day of incubation, assuming a constant EST throughout incubation (kindly provided by A. Lourens).

Besides embryonic heat production, EST is also determined by heat transfer. Heat transfer from the egg to the surrounding environment is affected by three main factors: 1) machine temperature, 2) air velocity and 3) relative humidity (Meijerhof and Van Beek, 1993; Lourens et al., 2011).

1) To maintain EST between 37.5 and 38.0oC throughout incubation, machine temperature need to be higher than 37.5 to 38.0oC during the first days of incubation (French, 1997; Lourens



et al., 2005, 2006a). In this period, the embryonic heat production is rather low and the egg is losing heat by evaporation. After approximately day 7 to 9 of incubation, embryonic heat production is sufficiently increased, meaning that machine temperature need to be gradually lowered to maintain EST. The level of heat production is affected by biotic factors, such as egg size (Lourens et al., 2006a), breed (Nangsuay et al., 2015), strain (Tona et al., 2010) and breeder age (Nangsuay et al., 2013) and consequently adjustment of the machine temperature to maintain EST depends on these factors.

2) Air velocity is of large importance for heat transfer from and to the eggs as well (Meijerhof and Van Beek, 1993; Lourens et al., 2011). In the first days of incubation eggs, need to be warmed and in the second half of incubation heat produced by the embryos need to be removed. When the air velocity within an incubator is low and variable, the difference between machine temperature and EST can be quite high (Lourens et al., 2011) and in that situation the variation in EST within an incubator will be high as well (Elibol and Brake, 2008). A high air velocity (up to 2 m/s) is needed to reduce the variation in heat transfer among eggs within an incubator and consequently in EST as well.

3) Heat transfer is also determined by relative humidity (Meijerhof and Van Beek, 1993; Van Brecht et al., 2005). Humid air transfers heat to a larger extend than dry air, meaning that heat transfer from eggs to the environment can be larger with a higher relative humidity. However, it need to be realized that increasing the relative humidity in the incubator by an humidifier can have negative effects. Eggs close to the humidifier will be cooled more by evaporative heat loss than eggs far away from the humidifier, resulting in increased variation in EST within an incubator (Meijerhof, 2009). Additionally, effects of relative humidity during incubation on hatchability and chicken quality are relatively small, when the EST in an incubator is maintained at a constant level of 37.5 to 38.0°C (Van der Pol et al., 2013).

#### **Consequences of incubation temperature**

Consequences of incubation temperature can be divided into two major categories: Effects on embryo mortality and chicken development at hatch and effects on later life performance. These aspects will be discussed below, including the proposed mechanism involved. Additionally, incubation temperature appears to have effects on specific organ systems as well, such as bone development and (intestinal) health. These aspects will be discussed as well.

#### 1) Effects on embryo mortality and chicken quality at hatch

In studies that used different EST during incubation most work is performed during late incubation. However, during the first week of incubation EST is important as well. Lourens et al. (2005) investigated effects of two different EST during the first week of incubation (36.7 vs 37.8oC). The lower EST in the first week resulted in a slightly higher embryonic mortality in the first week of incubation (10.2 vs 8.9%), more second grade chickens at hatch (3.8 vs 0.0%), shorter chickens at hatch (17.9 vs 18.8 cm) and a lower yolk free body mass (YFBM) at hatch (31.2 vs 34.6 g). So, a low EST during the first week of incubation can have considerable effects on chicken development. At the other hand, a too high EST (>40.0oC) during early incubation can results in increased mortality as well, particularly due to malformation, such as exposed brains, four legs and cross beaks (Wilson, 1991).

In late incubation, particularly negative effects are found of a high EST. The risk of high EST in

late incubation is considerable, mainly due to the high embryonic heat production in that stage. Lourens et al. (2005) compared an EST of 37.8 vs 38.9oC in the last week of incubation. At high EST, they found higher mortality in the last week of incubation (15.0 vs 8.6%), more second grade chickens at hatch (2.0 vs 0.0%), shorter chickens at hatch (18.2 vs 18.5 cm) and lower YFBM at hatch (32.7 vs 33.1 g). These results are supported by a recent study from Turkey, in which different ranges of EST were investigated from day 10 to 18 of incubation (Ipek et al., 2014; Table 1). This study clearly demonstrated that both a too high and a too low EST during the second stage of incubation resulted in higher mortality, particularly during the hatching phase (day 18 to 21 of incubation), more culls at hatch and lower YFBM at hatch. Among others, studies of Lourens et al. (2005) and Ipek et al. (2014) showed that chicken development is retarded by a slightly higher EST than optimal (37.5 to 38.0oC). Molenaar et al. (2010) again showed comparable results of a high EST (38.9 vs 37.8oC) between day 7 and 19 of incubation. Again the high EST resulted in higher mortality, particularly during the hatching phase. When a break-out was performed on the unhatched eggs, it was demonstrated that mortality particularly happened in the last few days of incubation and that more chickens demonstrated malpositions (head over wing, head between legs; 6.1 vs 1.3%), when exposed to 38.9°C compared to 37.8°C.

Table 1.	Effects of egg	gshell	temperature	during	day	10 t	o 18	of	incubation	on	mortality	and
chicken o	quality at hatch	ı (Ipek	et al., 2014)	).								

	, , ,		
EST day 10-18, °C	33.3-36.7	37.8-38.2	38.9-40.0
Hatch of fertile, %	84.2b	91.4a	83.0b
Mortality d 18-21, %	7.2a	1.6b	7.8a
Culls, %	2.7a	0.9b	2.7a
YFBM d 1 post hatch, g	31.5b	36.2a	33.4b
RY d 1 post hatch, g	5.1c	6.3b	7.7a
RY d 3 post hatch, g	3.2b	1.6c	4.8a

In a more practical setting, Elibol and Brake (2008) investigated effects of variation in EST within a commercial incubator on embryonic mortality. They assessed effects of egg weight at different places within in the incubator (near or far away from the fan) in relationship to embryonic mortality. As explained above, it can be expected that air velocity far away from the fan will be lower, meaning that heat transfer will be lower as well, resulting in higher EST. Because large eggs are producing more heat than small eggs (Lourens et al., 2006a), it can be expected that particularly large eggs far away from the fan will demonstrate higher EST, which might result in higher embryonic mortality. Indeed, large eggs (average 68.8 g) far away from the fan (38.7oC). This relatively small increase in EST resulted in higher embryonic mortality in the last week of incubation (6.9 vs 3.0%), lower hatch of fertile (81.7 vs 91.0%) and more second grade chickens (4.6 vs 1.2%). Altogether, it can be concluded that relative slight deviations from the optimal EST (37.5 to 38.0oC) in late incubation results in profound negative effects on hatchability and chicken quality at hatch.

In all studies indicated above, differences in EST were applied for at least one week, particularly during the developmental period (till day 18). In recent studies (Maatjens et al., 2014a, b) effects of EST during only the hatching phase (day 19 to hatch) on embryonic development were investigated. Although no effects on hatchability were found, a high EST (38.9oC) resulted again in the lowest YFBM at hatch compared to lower EST (36.7 or 37.8oC). Furthermore, strong negative effects of high EST were found on heart weight at hatch (see below).



#### 2) Effects of EST on later life performance

In case chicken quality at hatch will be affected by EST during incubation, it may be expected that later life will be affected as well (Decuypere et al., 2001). Only a few studies have investigated effects of incubation temperature during early incubation on later life performance. Joseph et al. (2006) investigated effects of a low EST during the first 10 days of incubation (36.6 vs 37.8oC) and reared chickens till day 42 of age. Body weight at day 42 of rearing (3014 vs 3103 g) and percentage breast fillet (23.2 vs 23.7%) were lower in chickens incubated at the low EST compared to the optimal EST.

More studies have been studying effects of high EST in late incubation on later life performance. Hulet et al. (2007) were one of the first that demonstrated that a high EST (39.7oC from day 16 of incubation to hatch) resulted in a lower BW at day 44 of rearing than an EST of 38.6oC in the given incubation period. Comparable results were found by Molenaar et al. (2011a). They exposed eggs to an EST of 37.8oC or 38.9oC between day 7 of incubation and hatch and reared chickens thereafter till 42 days of age. Chickens incubated at 38.9oC had a lower body weight at day 42 (2854 vs 2895 g), had a non-significant higher FCR (1.93 vs 1.91) and a higher mortality rate during rearing (12.5 vs 8.4%). When reasons for mortality were investigated, the difference in mortality between both incubation temperatures could be attributed to ascites (6.6 vs 2.8%).

# 3) Proposed mechanism involved in effects of high incubation temperature on chicken quality, growth performance and metabolic disorders

Based on the results indicated above, it can be concluded that particularly during late incubation a slightly higher EST than optimal results in lower chicken quality at hatch and impaired rearing results in later life. Although not all details are clear yet, the physiological mechanism involved becomes more and more clear. The hypothesized mechanism will be discussed below, with the focus on high incubation temperatures during late incubation.

Because the chicken embryo during late incubation acts as a poikilotherm, the body temperature and the metabolic rate depends on the EST (Lourens et al., 2006b). A high EST results in a high metabolic rate, meaning that more nutrients are required. Particularly, the glucose requirements for metabolism are increased (Molenaar et al., 2013). Because glucose storage in fresh eggs is very limited (<1%, Romanoff and Romanoff, 1949), an embryo will build up glycogen storages during incubation (Foye et al., 2007). During exposure to high EST in late incubation, oxidation of lipids from the yolk is reduced, because oxidation of lipids requires more O2 than oxidation of carbohydrates and O2 supply through the eggshell to the embryo is already limited during the plateau phase (Lourens et al., 2007; Molenaar et al., 2010). Consequently, less volk lipids will be oxidized, resulting in a larger residual yolk size at hatching (Lourens et al., 2005, Molenaar et al., 2010). Furthermore, the embryo will use the stored glycogen reserves, resulting in lower levels of glycogen, particularly in the liver (Molenaar et al., 2011b; Maatjens et al., 2014b). However, the stored glycogen is needed as fast available energy to be used during the hatching process (Freeman, 1969). In case glycogen is too much depleted due to high EST, the movement of the embryo at approximately day 17 from head between legs to head turned to the right and covered by the wing is disturbed, resulting in more malpositions and finally more dead-in-shell chickens.

Additionally, a chicken embryo will try to restore the glycogen storage by using gluconeogenesis (Molenaar et al., 2013). Because lipid oxidation is retarded (see above), glycerol availability

as precursor for gluconeogenesis is reduced. The embryo will use glucogenic amino acids to produce glucose. Glucogenic amino acids will be obtained from the chicken body (YFBM), because at that stage of incubation almost all protein from the egg is already used. When amino acids will be obtained from the body, proteins from muscles will be broken down, resulting in a lower YFBM (Lourens et al, 2005, 2007; Molenaar et al., 2010, 2011b, 2013; Figure 2). Additionally, an important muscle is the heart and it has been shown that a high EST during late incubation indeed results in an up to 30% lower heart weight at hatch (Wineland et al., 2000; Lourens et al., 2007; Molenaar et al., 2011b; 2013; Maatjens et al., 2014a). That body protein is broken down is also demonstrated by the higher uric acid levels at hatch in chickens exposed to high EST (Molenaar et al., 2013). It can be imagined that a chicken with an up to 30% lower heart weight at hatch will have more difficulties for sufficient oxygen supply at tissue level when metabolic rate is high in later life. Molenaar et al. (2011a) demonstrated an enlarged right heart ventricle at day 42 of age in chickens that were exposed to high EST between day 7 and 19 of incubation suggesting that retarded heart development at hatch still have effects at slaughter age of chickens. The development of metabolic disorders, like ascites, can be increased in case the heart size already at hatch is retarded.



**Figure 2.** Effect of normal (37.8oC) or higher (38.9oC) eggshell temperature (EST) from day 7 to 21 of incubation on yolk free body mass (YFBM) of broiler chickens (Molenaar et al., 2013).

#### 4) Bone development

Several studies have investigated effects of incubation temperature on different aspects of bone development. Yalçin et al. (2007) were one of the first investigators demonstrating that incubation temperature affected bone development and the incidence of tibial dyschondroplasia (TD). Both a low (36.9oC) and high (39.6oC) incubation temperature during day 0 to 8 or day 10 to 18 of incubation resulted in lower tibia weight at day 14 of incubation and at hatch compare to the control incubation temperature of 37.8oC. Furthermore, both the low and high incubation temperature during early incubation resulted in long term effect, demonstrated as a higher tibia ash content at day 49 of rearing and a higher incidence of TD at the same day. Recent work of Van der Pol et al. (2014) demonstrated negative effects on bone development (femur, tibia, metatarsus) when an EST of 39.4oC was provided throughout incubation compared to



36.9, 37.8 or 38.6oC throughout incubation. This is in agreement with Oviedo-Rondón et al. (2008), who found shorter tibia, femur and shank lengths in turkeys at hatch, when they were incubated at 39.0oC from day 24 of incubation to hatch onward compared to 36.0, 37.0 or 38.0oC. In both 38.0 and 39.0oC treatments, relative asymmetry was increased, which might be an predictor of locomotion problems and TD in later life (Yalcin and Siegel, 2003). In another study with broilers, Oviedo-Rondón et al. (2009) investigated effects of both low (36.7 vs 37.5oC) incubation temperature between day 1 and 7 of incubation and high (39.0 vs 37.0oC) incubation temperature between day 18 and 21 of incubation. Although effects on leg bones at hatch were not clear, at day 41 of rearing, chickens incubated at a high temperature during the last day of incubation demonstrated (numerically) more crooked toes (14.3 vs 9.7%), more footpad dermatitis (3.3 vs 1.6%) and more hockburns (54.3 vs 44.8%). Additionally, gait score at day 41 of rearing was retarded with a high incubation temperature in late incubation. However, not all studies are unambiguous. Shim and Pesti (2011) did not find any effect of incubation temperature during day 4 to 7 of incubation on bone development, whereas Hammond et al. (2007) found longer femur and tibia in layer chicken embryos at an incubation temperature of 38.5oC between day 4 and 7 of incubation compared to an incubation temperature of 37.5oC in that period. It can be suggested that effects of incubation temperature on leg bone development depend on the period incubation temperatures are applied, the used incubation temperature and probably breed, strain and age of the breeder flock.

#### 5) Intestinal health

Relationships between incubation temperature and intestinal development and health are scarce, but in some studies in the last decade, some indications are given that incubation temperature could play a role. In layer chickens, Walstra et al. (2012) demonstrated that chickens incubated at a low EST (36.7oC) in the first week, combined with a high EST (38.9oC) in the last week of incubation chickens tended to be more vulnerable for *Eimeria acervulina* inoculation at day 36 of rearing than chickens incubated at a constant EST of 37.8oC throughout incubation. This was expressed in a tendency to lower feed intake and body weight gain after inoculation, more duodenal lesions and higher oocyst production after inoculation with Eimeria. These effects might be due to effects of incubation temperature on intestinal morphology and expression of nutrient transporter mRNA in different parts of the intestine as demonstrated by Barri et al. (2011). Another explanation might be that incubation temperature affects the development of bursa of Fabricius and thymus follicles. Oznurlu et al. (2010) demonstrated that an incubation temperature of 38.8oC (resulting in 40.1 to 40.6oC EST) from day 10 to21 of incubation retarded the development of these follicles compared to an incubation temperature of 37.8oC in that period. Additionally, peripheral lymphocyte levels were lower in the chickens incubated at the higher temperature. This all suggests that a high incubation temperature in the second half of incubation seems to have health consequences in broiler chickens, which might have consequences for the adaptive capacity of the chickens in later life.

#### Conclusion

It can be concluded that incubation temperature has strong effects on hatchability and hatchling quality, but moreover relative small differences in incubation temperature can have large effects in later life in several organ systems. Because of that it can be advised to control eggshell temperature instead of machine temperature, because at a given machine temperature, eggshell temperature can largely vary.

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# O<sup>03</sup> Effect of Using Cardboard or Plastic Viols on Hatchability

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### Abstract

This experiment was conducted to determine the effect of using cardboard or plastic viols on hatchability. Hatching eggs were obtained from the broiler breeder flocks aged 30 wks (young) and 54 wks (old). Plastic viols adapt to the air temperature much uniform than cardboard (p<0.01). Crack eggs percentage was significantly lower in cardboard in older flock eggs compared to plastic viols. However hatch of fertile eggs were improved in older broiler breeder due to lower early embryonic mortality in plastic viols (p<0.05), whereas in young flock there was no effect of viol types on percentage of crack eggs and hatchability (p>0.05).

Key words: Broiler, egg collection, plastic viol, cardboard viol, hatchability.

# O<sup>04</sup> Comparison of Some Production Characteristics of the White and Bronze Turkeys *(Meleagris gallopavo)* Under Intensive and Semi-Intensive Conditions, I. Growth Performance

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### Abstract

This study was conducted to investigate the effects of genotype, sex and different feeding types on growth performances of turkeys. The study population containing White and Bronze Turkey poults was sheltered at completely closed and controlled compartments during an eight week period regardless of the sex and feeding type. The live weights of the turkeys on the 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> days were determined by fixing with interpolation.

At the end of the eight week period, White and Bronze turkeys were found to reach average live weights of 1711.44 g and 1318.08 g, respectively. Following the differentiation according to sex and feeding type, the average live weights of male-female White turkeys and male-female Bronze turkeys belonging to intensive group were calculated as 6205.9-4750.0 g and as 5258.0-4112.9 g, respectively, at the end of the study period prior to slaughtering. On the other hand, the average live weights of male-female white turkeys and male-female bronze turkeys belonging to semi-intensive group were determined as 5631.8-4577.7 g, and as 5028.9-4133.0 g, respectively. The differences between the sex and feeding type groups in both genotypes were found to be statistically significant (P<0.001).

The effects of sex and feednig type on both genotypes were found to be statistically significant (P<0.001). Total feed consumption was determined to be higher in males than females and in intensive group than semi-intensive group in both genotypes (P<0.01, P<0.001).

The present study aimed to investigate the feasibility of intensive turkey breeding which still lacks sufficient attraction, and the results suggest that an integration-like model which is widely applied in broiler production and marketing can also be used in turkey breeding. This may be beneficial as an alternative poultry breeding. It is concluded that breeding of Bronze Turkeys under semi-intensive system and of white turkeys under intensive system might be more convenient.

Key Words: White turkey, bronze turkey, intensive, semi intensive, growth performance



# O<sup>65</sup> EU Regulatory Environment For Feed Additives Involving GMOs

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Since the 80s, the development of the techniques of genetic modification has started and already by the beginning of the 90s, the European Union (EU) authorities have initiated the first regulations of these products. The regulatory system established a mandatory evaluation system for Genetically Modified Organisms (GMOs) used in plain field, particularly focusing on their impact on the environment<sup>1</sup>. For Genetically Modified Microorganisms (GMMs) used for industrial (fermentative) production processes, the control focused on the containment measures linked to the safety profile of the microorganisms. A system of site authorization was also established<sup>7</sup>.

At the beginning of the current century, as a follow up of different food safety issues in the EU, a full set of regulations was installed with a view to increase the safety of food for human health, animal health and the environment. Within this set of regulations, the Feed Additive regulation<sup>4</sup>, published in 2003, sets up a pre-market authorization system, based on a thorough evaluation of the safety of feed additives. For products produced by fermentation using a GMM, the risk evaluation is based on a thorough description of the production organism and of its genetic modification process (as described in the guidelines for the preparation and evaluation of application dossiers<sup>6</sup>). Thus, feed additives obtained by fermentation are only authorized if the risk evaluation confirms that the production strain and the end product (the feed additive) are safe under the defined conditions of use.

In parallel and linked to the development of GMO used in plain field, the EU authorities have developed a set of regulations (GM regulations<sup>2,3</sup>) aiming at further ensuring safety of GMOs and their products in the food and feed chain. Considering that the feed additives produced by fermentation with a GMM were already evaluated and that the final product is sufficiently purified, the regulators excluded them, when purified (e.g. enzymes, amino-acids, vitamins,...), from the scope of the GM Regulations<sup>2,5</sup>. Microbial biomass recovered from these processes, consisting of the killed cells of the micro-organisms, where DNA is no longer transferable, and which are intended to be used as feed materials are covered by the scope of the GM regulations. Furthermore, with a view to provide the consumers with the freedom of choice based on relevant information, the GMOs and their products need to be labelled with their GM origins (e.g. oil produced from genetically modified soybean, betaine produced from GM sugar beet, etc.).

The overall regulatory system of Genetically Modified (GM) Food and Feed therefore ensures that all products produced from or with the help of GMO (or GMM) are thoroughly evaluated by the European Food Safety Authority (EFSA). In addition, a labeling system based on full traceability has been established for the products containing or consisting of GMO, as well as for products that involve the use of GMO in plain field (e.g. oil produced from GM soybean).

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- 2. Regulation (EC) No 1829/2003 on genetically modified food and feed
- 3. Regulation (EC) No 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC
- 4. Regulation (EC) No 1831/2003 on additives for use in animal nutrition
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# O<sup>66</sup> Amino Acids Ensure Sustainable Feed and Livestock Production

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### Introduction

The world and its inhabitants face many challenges ahead. Population growth is rapidly expanding, and it is expected that we will reach a global population of 9 billion people by 2050. This population expansion is accordingly putting enormous pressure on our natural resources. Today, we can no longer debate if the inhabitants of earth are impacting its resources, but rather we must focus on how to sustain higher living standards for an increasing world population while dealing with less natural resources and more climate change. To do this, one must quickly realize that these social, environmental, and economical issues are not independent, but rather co-dependent as shown in Figure 1.

### Figure 1. Sustainability Model



Source: Wikipedia

While there is growing sentiment for climate protection, the world population is steadily growing and thus requiring a sustainable supply of high quality and safe food. To further complicate this, the growing population is also becoming more affluent, especially in developing countries. The direct result is increased demand for meat and milk products, which further intensifies the discussion on climate change.

Ehrlich and Holdren (1974) introduced a simple equation to describe the relationship between environmental impact, population size, consumption level and technology. Their equation: I = P x A x T where:

I = environmental impact; P = population; A = affluence (consumption); T = technology Basically stated asks: "what is the environmental impact, given the relationship between population size, consumption level (affluence) and technology?" Animal agriculture is a major player in these global environmental issues. The huge demand for feed crop production shapes entire landscapes and can reduce natural habitats, causing degradation in some areas. The objective of this abstract is to paramount the role of poultry production against the background of this sustainability challenge. Further insight shall be given into how technology, such as the use of supplemental amino acids, can benefit the long-term poultry production, our environment and finally world's population.

### Technology is a key driver of global animal production

Technological improvement is a key driver of global livestock production. Growing productivity has been achieved through advanced breeding and feeding technology. The use of concentrate feed, more productive breeds through better genetics, animal health improvements and developments in the post–harvest sector can also be counted as technological improvements

A theoretical example highlighting the importance of technology is provided in Table 4. This example shows that a high consumption level per capita is not necessarily a negative as it also depends on technology and on the number of people. A small population with a high consumption level but under-developed technology can cause much more environmental damage than a very large population with medium size consumption level but progressive technology. It depends on the combination of these variables and technology is the critical factor. Although consumption is doubled, the impact on the environment can be kept constant or reduced by improving the technology.

 Table 1. Influence of technology on relative environmental impact is demonstrated in this theoretical calculation

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Р	Population	100	100	100	100	100	100	100
Α	Affluence	5	5	5	10	10	10	10
Т	Technology	1	0.5	0.1	1	0.5	0.1	0.05
Ι	Impact	500	250	50	1000	500	100	50

### Reducing the impact of poultry production

Globally, around 9 % carbon dioxide, 35–40 % of methane, 65 % of nitrous oxide and around 64 % of ammonia are derived from animal production. Animal production also has an enormous impact on water use as illustrated in Table 5. Advanced nutrition can alter the impact of poultry production significantly in these respects via balanced feeding strategies. As a general rule, a one percentage point reduction in dietary protein will lead to reductions of 10 % nitrogen in manure, 10 % ammonia emissions into the air, 3 % water consumption and 5 % manure volume.

Due to the increasing demand for meat, milk and eggs, it is unlikely that there will be an alternative to intensive livestock production. Therefore, we must look at how to mitigate or minimize the environmental impact in high density livestock areas.

To further illustrate how improving feed efficiency and reducing the nutrient excretion can help mitigate the overall impact of poultry production, a life cycle assessment (LCA) for a typical broiler production scenario can bused. In general, life cycle assessments describe the complete fate of a product by compiling and evaluating all ecological input and the consequences for the environment during each phase in the life cycle of the product based on international



standards (DIN EN ISO 14040/44:2006) . This covers the production of raw materials for the manufacturing process, the use by the consumer, and including the disposal of the used product.

But such an LCA reflects only one exemplary feeding scenario. To demonstrate the sustainability improvement potential of each feed formulation, a new web-based system AMINOFootprint® is now available to assess the ecological footprint of each individual poultry diet. AMINOFootprint® is a web-based application for notebooks and tablets. It focuses on calculating ecological profiles of compound feed and enables the identification of diets with the least environmental impact. This is a change within the feed industry. Optimizing the nutritional and economic dimensions of compound feed has always been core to the added value that feed additive companies promise to deliver; however. Now diet evaluation will be based on a third dimension as a broad approach to sustainable diets.

As already expressed through the equitation of Ehrlich and Holdren (1974) the reduction of crude protein in diets for livestock production leads to environmental improvements. This effect can also be easily shown when calculating and comparing the ecological profiles of diets due to their specific crude protein content. Table 2 below describes typical diets for broilers where the level of crude protein is stepwise reduced by replacing soybean meal with additional amino acid supplementation. The impact to the specific contributions to the Global Warming Potential (GWP expressed as kg  $CO_2$ -eq/mt feed), Acidification Potential (AP expressed as kg  $SO_2$ -eq/mt feed) and the Eutrophication Potential (EP expressed as kg  $PO_4$ -eq/mt feed) are shown in figure 2 including the use phase and animal performance in a so-called "Cradle to Grave"-approach including the nitrogen digestibility of the animal and thus the impact of the nitrogen excreted with the manure.. Figure 2 furthermore illustrates the specific improvement potential for the environmental performance of broiler production following the advanced feeding technology of amino acid supplementation.

Ingredient	Diet A Share [%]	Diet B Share [%]	Diet C Share [%]
Soybean Meal (48%)	29,92	26,63	23,26
Corn	65,31	37,99	10,73
Wheat	0	30	60
Soybean Oil	1,25	1,81	2,34
Biolys	0,09	0,23	0,28
DL-Methionine	0,13	0,13	0,14
L-Threonine	0	0	0,04
Mineral Premix	3,3	3,21	3,21
Crude Protein Content	19,6	19,6	19,4

The reduction of the crude protein content by replacing imported soybean meal through locally produced cereals leads to a stepwise reduction of the GWP up to roughly 140 kg  $CO_2$ -eq/mt feed, the AP 4,5 kg  $SO_2$ -eq/mt feed and of the EP contribution by 1,2 kg  $PO_4$ -eq/mt feed.

 Table 2: Composition of different broiler diets, where Soybean meal and corn are stepwise replaced by wheat for further calculations with AMINOFootprint®

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**Figure 2**:Ecological improvement potential of the typical broiler diet in table 2. The crude protein content (CP) is stepwise reduced to show the ecological improvement potential on the Global Waming potential (GWP expressed as kg  $CO_2$ -eq/mt feed), Acidifcation Pontential (AP expressed as kg  $SO_2$ -eq/mt feed) and Eutrophication Potential (EP expressed as kg  $SO_4$ -eq/mt feed) calculated with AMINOFootprint<sup>®</sup>.

### Conclusions

Low protein diets contribute to reduce the impact of pig production on climate change, acidification and eutrophication. As for current feeding practices, there is still a major potential to mitigate this impact. This potential is highest when SBM originating from plantations is replaced by a combination of more feed amino acids and alternative feed raw materials.

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# O<sup>07</sup> Challenges of Animal By Products in Turkey and EU Perspective

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### Introduction

Approximately 30 to 50% part of the slaughtered animals which are raised for their animal products such as milk, meat and eggs are not consumed by human beings and evaluated as animal by products. After slaughtering of the animals, offal's are being processed to obtain meat and bone meal, poultry rendering by product meal, feather meal, blood meal, fish meal, tallow, poultry rendering fat in abattoirs or rendering plants. Some of the animal by products are further processed and converted to drug and cosmetic products.

The importance of these animal by products for the feed industry stems from their high amount of animal protein, essential amino acids, calcium, phosphorus and metabolic energy contents. These attributes make animal by products essential constituents for poultry, pig, fish and pet animals' feeds.

Nevertheless, feeding to ruminants any of the animal by products obtained from ruminants was banned after Bovine Spongiform Encephalopathy (BSE) incidents showed up in 1996. Upon BSE crisis significant changes were made in existing feed laws and new regulations have been amended in EU, USA, Turkey and many other countries. In addition to this feeding of rendering products were severely restricted or completely banned within the same species of animals in EU on the grounds to avoid cannibalism. Especially the last precaution put a heavy of burden on the feed industry because of the ban on these valuable animal protein sources as feed ingredients. Balancing the feeds in terms of essential amino acids and calcium and phosphorus have become difficult. In this regard ethical and scientific approaches to the application of animal by products into animal feeds except ruminants have to be differentiated and revised if necessary.

In Turkey the ban/restrictions date for the use of animal by products in animal feeds is 01/01/2016 and envisaged sanctions will be put in force starting from this date. Officials, research institutions and sectoral stakeholders should focus on the issue to reevaluate the matter. The existing rendering plants should be revised from quality and quantity standpoint and if necessary new technologies should be introduced in order to produce high quality and healthy animal by products. This is essential for compliance with the EU rules and requisite for productivity and decreasing the production costs as well.

### **History of Rendering Products**

From historical perspective animal by products were obtained in the very old time and they are as old as history of humanity. Before agricultural era human beings were hunter's gatherers and they used to nourish with flesh of animals from which they hunt and made use of horn, nails, hide, leather, tail and bones of the animals by making various equipment, weapons, attire, and shelters.

Early records concerning animal by products are belong to 2000 years ago. Animal by products



were processed at that time. The soap that was used as drug and a cleaning product during Roman Era was obtained from goat tallow and wood ash through rendering process.

Jabir Ibn Hayyam known as father of chemistry who lived I 8<sup>th</sup> Century, stated that "soap is the most important cleaning item". Soap which has been produced in Marseilles could only be used by wealthy class until modern age.

Professor C.S. Plumb from Purdue University in 1901, probably inspired by European animal nutritionists, incorporated animal protein (tankage) in pig feeds which normally used as soil manure: By feeding pigs with Tankage + Maize mixture, fattening period reduced from 9-12 months to 7 months. It was understood that tankage played a crucial role in shortening of the fattening period as an important protein source.

Agricultural industrialization was pioneered by Industrial revolution of the 19<sup>th</sup> century, proliferation of intensive farming in livestock sector, increase in farm size, and increase in large capacity abattoirs establishment call forth significant need of offal or waste disposal. Thus, rendering plants were established and new technologies were developed and implemented to solve this offal disposal problem. This progress helped resolution the problem by processing these offal in rendering plants, convert them into valuable healthy animal by products and finally overall recycling of these waste could be made possible. Hereby possible environmental challenges could be prevented through rendering technologies.

Some of the animal byproducts have become main raw material of some of industrial products. For example, glue was invented using bounding property of fats and proteins obtained from animal by products. Glue produced from an animal byproduct, collagen has made a pick by reaching 70 million kg in 1948.

### Radical Changes in the EU Feed Law and Regulations (EC 1774/2002)

Further industrialization in animal husbandry and enormous increase in production seen in 1980s and in 1990s caused environmental crisis which ended up in livestock and food industries severe devastations. Primarily BSE, Dioxin, Foot & Mouth Disease (FMD) and Genetically Modified Organisms (GMO) crisis have global impact on feed and food sectors and adversely affected these industries.

Significant changes have been made in the feed regulations of prominent countries such as USA, EU, Canada, Japan, Turkey and many other countries due to the environmental and health crisis indicated in above encountered in Livestock industry. The EU Feed Law and Regulation has been reshaped and amended entirely by putting into place a series of directives (EC 1774/2002; EC 1069/2009; EC 749/2011; EC 142/2011) based on the security terms, principals and lessons learned from BSE, Dioxin, FMD, GMO experiences. After a long time period in Turkey Veterinary, Plant Health, Food and Fed Law and Regulations (5996/2010) and "Animal by products not intended for human consumption" Directive (24/12/2011) and Biosecurity Law and Regulations (5977/2010) were amended and put in force in compliance with EU regulations.

### **Animal Byproducts Categories**

Animal by products have been divided into basically 3 categories:

Category 1: Animal by products with Bovine Spongiform Encephalopathy (BSE) risk, unknown

risks or animal byproducts contaminated with toxic substances or environmental pollutants. **Category 2**: Animal by products with risk of animal diseases or with risk of veterinary drug or chemical residues.

**Category 3**: Animal by products obtained or processed from pre or post slaughtered healthy animals.

Animal by products can be use or disposed according to their categories as indicated in Table 1.

	Rendering	Incineration	Pet food	Compost	Biogas
Category 1	Yes	Yes	No	No	No
Category 2	Yes	Yes	No	No	No
Category 3	Yes	Yes	Yes	Yes	Yes

 Table 1. Use and Disposal of Processed Animal Products

### Bans on Animal Byproducts in the Feed Industries of EU and Turkey

- Animal by products or processed proteins obtained from a terrestrial species cannot be fed to an animal in the same species. The same rule is applied to fish species. That means poultry rendering byproduct meal cannot be fed to poultry of the same species. In fishes, trout meal cannot be fed to salmon for instance (they both belong to the same species) and neither pig meal can be fed to pigs. Fur animals are exempt from this rule.
- 2) None of ruminant or any other animal by products or processed protein from an animal can be fed to ruminants (cattle, sheep, goat and, etc.).
- 3) In EU several research and methodologic studies have being conducted in order to feed animal by products in reciprocal manner between pig and poultry. However these efforts, so far have not been resulted in success.
- 4) The rule indicated in (1) have been postponed until 01/01/2016 in Turkey in compliance with adaptation of EU regulations. However, in Turkey the feed and poultry industries do not seem to be ready to implement this rule.

### Conclusions

- In Turkey from the end of 2015 and onwards approximately 1.2 million tons of animal offal and waste disposal will cause serious environmental challenges,
- In Turkey availability of sufficient infrastructure waste disposal in terms of rendering and incineration plants is doubtful,
- Is there species specific methodological validation in our reference labs?
- 539 000 tons of rendering animal by products will not be used as animal feed (except very limited amount might be incorporated in fish feed).
- To fill the gap of this ban 700 000 tons of soybean meal will have to be imported.
- Feed industry will have import significant amount of dicalciumphosphate to balance Ca and P requirement of the animals, however this means more pollution of soil and water.



	Amount (metric tons)	Value (TL/metric tons)	Total Value (million TL)
Poultry by product meal	228.981	2.000	458
Poultry fat	72.310	2.000	145
Total poultry rendering products	301.291		603
Meat and bone meal	189.292	1.200	227
Tallow	48.851	2.000	98
Ruminant rendering products	238.144		325
Total Rendering products	539.435		927

### Table 2. Rendering Products Amount and Value in Turkey (2015)

### Suggestions

The provision in "Animal by products not intended for human consumption" directive article: 48 item: (a) "...ban of feeding poultry rendering by products to poultry within the same species...";

- Should be suspended for 10 years or,
- Should be suspended until the date of Turkey's full membership to EU
- Support for poultry industry to establish waste disposal plants and facilities indicated in Category 1 and 2 wastes.

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# O<sup>08</sup> Food Safety Based on Risk Analysis in Poultry Meats

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#### Abstract

The safety of poultry meat and products had been evaluated as a health, economic and social importance for many years and seems to extend this situation in the future. In the basic manner poultry meat hygiene consists of ante-mortem and post-mortem inspection, monitoring and surveillance (microbiological and chemical hazards) with hazard analysis and critical control point (HACCP), good manufacturing practices (GMP), good hygiene practices (GHP) at every stage of production. Therefore all of these applications have an impact on poultry meat safety for human consumption. For achieving real poultry meat safety "farm to fork" concept have to be set up with traceability of product under risk analysis approach. Risk analysis consists of three elements; risk assessment, risk management and risk communication. Official authority, university, research centers, industry and society have to establish consciousness as they are the stakeholder of this fact.



# O<sup>09</sup> Effect of Cooking Methods (Gas and Electric Oven) and Times on Heterocyclic Aromatic Amines (HAAs) Formation in Chicken Döner

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### Abstract

The study was carried out to determine the effect of varying degrees of donenes (rare, medium, well done), different cooking methods (gas and electric oven) and different döner types (yaprak and karışım) on Heterocyclic Aromatic Amines (HAAs) formation in chicken döner which has an important place in fast-food sector. Within this scope, nine common HAAs were investigated: IQ, IQx, MeIQ, MeIQx, PhIP, 4,8-DiMeIQx, 7,8-DiMeIQx, A $\alpha$ C and MeA $\alpha$ C. In addition, creatine, creatinine, total free amino acids, and glucose were analysed as HAAs precursors. 4,8-DiMeIQx, PhIP and MeIQ were the most dominant HAAs in chicken döner while MeIQx and A $\alpha$ C were the least HAAs. MeA $\alpha$ C was not detected (ND) in any of the samples. When comparing creatine, creatinine, total free amino acid, and glucose contents of raw meat with those of cooked meats, creatine content decreased while creatinine content increased due to cooking (p<0,05). In addition, total free amino acid and glucose contents also decreased as a result of degredation or reaction with other components to form HAAs.

**Key words:** *Chicken döner, heterocyclic aromatic amines, cooking methods, cooking time, food safety* 

# O<sup>10</sup> Broiler Chicken Pectoral Myopathies

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Since the 1950's, intensification of breeding efforts has been extremely successful in the introduction and widespread utilization of meat-type (i.e., broiler) strains of chickens for commercial poultry meat production. Heritable performance traits, such as growth rate, efficiency of feed conversion, weight uniformity, general disease and colonization resistance (i.e., livability), meat yield, tissue integrity and functionality (skin, skeletal, digestive, muscular and immune systems), and body composition (feathering; meat to bone ratio; fat content and distribution) have facilitated the development of strains of broiler chickens to fulfill the quality demands of rapidly expanding animal protein market. Whether marketed as whole or portioned, boneless-skinless, or further processed, poultry meat products must consistently meet the quality of demands (yield, grade, color, portion size and shape, composition, safety and wholesomeness) expected from a myriad of ready-to-cook and ready-to-eat products.

The selection emphasis on economically important white meat (i.e., pectoral muscles) accretion and yields has been particularly effective, as the two pectoral muscles (fillet and tenders) now constitute nearly 1/3 of the live weight of a broiler chicken. Such an improvement in breast muscle development has not been without concomitant changes in muscle structure, metabolism and functionality. Pectoral muscles of modern strains of broiler chickens are now almost entirely composed of hypertrophic, tonic and fast-twitch fibers, with potentially marginalized vascular supply.

Several pectoral muscle abnormalities have been recognized in broiler chickens, including those due to genetics (inherited muscular dystrophy), nutrient deficiencies (exudative diathesis), and toxicities. However, recently high incidences idiopathic myopathies affecting both the minor (Deep Pectoral Myopathy) and the major pectoral muscles (White Striping, Woody Breast, Necrotic Fillets) have been observed under commercial conditions. These idiopathic myopathies have a range of implications for the broiler industry both from a product quality and animal welfare standpoints.

### **Deep Pectoral Myopathy:**

Deep Pectoral Myopathy (or Green Muscle Disease) basically refers to the necrosis of minor breast muscle (i.e., tenders) of poultry. The condition was first recognized in turkeys, but has been detected in spent broiler breeders and more recently in heavy broiler chicken flocks. The necrotic tenders may appear swollen or dry-friable and often range in color from pale (ischemia) with bloody surface (early stages) to yellow-green (24 h or older). The lesions are usually limited to the middle portion of the affected muscles and are undetectable on whole carcass and cut-up parts (unless de-boned), although sunken appearance of the breast is reported in older birds.

### White Striping:

White striping (WS) refers to the presence of white striations that run parallel to the direction



of muscle fibers primarily at the thick end of the broiler breast fillets. Such striations have also been observed in thigh muscles. The white striations can exceed 1 mm in diameter and extend the whole length of the fillet, especially in heavy broilers or those experiencing rapid growth rate. Affected fillets have been shown to contain higher fat and lower protein levels. In fact, the striations are often characterized histologically as replacement (i.e., reparative response) of damaged muscle fibers (due to growth-related strain and overstretching) by fatty degeneration and fibrotic material. White striping can negatively affect consumer acceptability, especially when its severe. In addition, affected fillets exhibit lower marinade uptake, higher purge and cook loss.

### Woody Breast:

This myopathy primarily affects the *Pectoralis major* (I.e., fillets) muscles. The affected breast fillets exhibit focal or diffuse pale areas (ischemia) either completely hardened (dense) and bulging to the touch or show hardened "ridges" upon palpation, especially along the ventral portion of the breast. The surface of the fillets may contain a viscous-gelatinous exudate with or without petechial hemorrhages. The woody breast (WB) may be histologically characterized as an aseptic severe degenerative myopathy.

### **Necrotic Fillets:**

This necrotic myopathy (NF) perhaps represents the final stage of the progressive degeneration of the breast muscle of broiler chickens. Surface of the muscles exhibit scattered hemorrhages (especially at the cranial/shoulder end of the fillets) of varying severity with an accompanying gelatinous, often bloodstained, exudate. Usually the entire muscle is affected. No evidence of external trauma is seen, consequently the lesions may only be observed upon removal of the skin over the edematous tissues.

### **Histopathology:**

Histologically, degenerative changes characterizing these myopathies resemble those typical of idiopathic myopathies with a multitude of cellular lesions (i.e., variable fiber size; scattered focal necrosis; hyalinization and hypercontraction of muscle fibers; fragmentation; infiltration of the loose interstitial tissue by inflammatory cells, especially macrophages and heterophils; fibrosis and fatty tissue deposition) varying depending on the myopathy. Hard texture typical of WB results from excessive accumulation of extracellular matrix (mostly collagen and fibroplasia).

### **Etiology:**

Spontaneous or idiopathic myopathies have been correlated with enhanced growth rate and muscle accretion in meat-type broiler chickens. Pectoral muscles of broiler chickens are used for the upward and downward movements of wings and are uniquely composed entirely of glycolytic (anaerobic) myofibers that contract fast but fatigue easily. Although pectoral muscles are no longer used for flight in domesticated poultry, they play an equally important role in balancing and locomotion. Post-hatch muscle growth in poultry is driven primarily by hypertrophy of muscle cells from protein accretion. In genetic strains selected for rapid growth and high meat yield, the enlargement of myofibers may result in lower capillary density thereby potentially limiting the supply of nutrients and oxygen as well as the removal of metabolic by-products (lactic acid). This process is likely to be marginalized under exertion of pectoral muscles. Given this unique structure, function and metabolism, the hypertrophic pectoral myofibers may outgrow their support systems, such that susceptibility to damage is enhanced, especially with rapid rate

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of growth, management practices that limit muscular activity, and under thermal stress. Muscle damage can occur even in the absence of growth, mobility or postural problems in poultry. In the case with DPM, the ischemia of the minor pectoral muscles is triggered simply by exertion and self-strangulation from the sustained increase in intra-fascicular pressure. Broiler chickens spend most of their time resting with their pectoral muscles in full contact with the flooring/bedding. Focal or diffuse ischemia can result from transient or prolonged compression of vascular supply and/or from vascular thrombosis (i.e., intravascular coagulation due to stagnation of blood flow) in pectoral muscles. Current state of knowledge on muscle damage, repair and fibrosis development is based on extensive research in human muscular dystrophies. Physiological studies indicate that irreversible muscle cell damage can occur even after 3 hours of myo-ischemia. In addition to transient and prolonged ischemia, cellular/membrane damage can also be triggered by disturbances in intracellular cation regulation, alterations in acid-base balance and by production of reactive oxygen species. Loss of membrane integrity then leads to the leakage of inflammatory factors, such as proteolytic enzymes, angiogenic factors and fibrogenic cytokines in muscles. Additional inflammatory cells are attracted to the affected areas to phagocytose cell debris. Satellite cells that usually lie quiescent among the muscle fibers are activated to proliferate to repair the damaged tissues. In addition, fibroblasts attracted to the area proliferate to produce new extracellular matrix components. If the normal muscle repair and regeneration process fails, then the fibrotic scar is infiltrated by adipocytes (fatty degeneration). As compared to mammals, these inflammatory events occur extremely quickly in birds.

### **Recent Research:**

Research conducted at Auburn University on broiler chicken pectoral myopathies indicate that the incidence and severity of WS, WB and NF in broiler chickens are observed in all commercially available genetic strains of broilers, at varying slaughter weights (high>low), and by sex (males>females). Low genetic heritability values and genetic correlations with slaughter weight and breast yield indicate a polygenic nature of these myopathies. Therefore, contributing environmental/management factor(s) must be identified. For example, high muscle accretion rates that result from feeding diets high in amino acid density increase the incidence and severity of WS, WB and NB. Conversely, a reduction in feed intake, either voluntarily due to heat stress or by quantitative allocation, lowers the severity of myopathies and corresponding plasma levels of creatine kinase (CK) and lactic dehydrogenase enzymes (common indicators of muscle damage). Broiler chickens normally exhibit elevated levels of plasma CK, most likely due to high protein turnover associated with muscle tissue growth. Additional research is warranted and is being conducted to define primary the trigger(s) of these myopathies in meat type chickens.

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# O<sup>11</sup> Ventilation Basics For Modern Broiler Housing

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### Introduction

Good ventilation is one of the most important parts of good broiler production. In cold weather, ventilation plays a major role in air quality and temperature regulation. In warm and hot weather, ventilation is the major tool for removing excess heat from the house and the birds. Worldwide a large percentage of broiler housing utilizes the concept of negative pressure. This is in contrast to natural or curtain style ventilation. Managers and growers still struggle with understanding the basics of negative pressure ventilation and how it can be such a powerful tool for increasing performance and profitability. Basic concepts and principles to help in that understanding are presented in this paper.

### Ventilation Goals

Except with very young birds and/or very cold weather, temperature control is one of the primary goals of ventilation. At each stage of a bird's development, there is one optimum performance temperature zone at which the bird makes best use of feed energy for growth, as shown in the diagram below. Ventilation prevents heat build-up and keeps birds in this optimum performance zone by exhausting warm air from the house, by effective cooling through tunnel ventilation, and by lowering the actual air temperature through evaporative cooling. The target temperature for best broiler performance changes during a growout, typically from around 90°F on day one to near 70°F or lower just before catch, and ventilation must be adjusted accordingly to maintain temperature on target and assure good air quality.

High humidity is most often a winter problem and can affect bird health. Even when ventilation is not needed for heat removal, we must maintain at least a minimum rate of ventilation to prevent wet, caked litter, ammonia problems, and other air quality problems.

Ventilation to provide fresh air is needed in all seasons and in hot and cold weather.

The flip side of bringing fresh air in to replenish oxygen is getting rid of the birds' "exhaust fumes," mainly carbon dioxide. The most common toxic gas problem, however, is ammonia coming from too-wet litter, which leads to health problems and lowered performance. Proper ventilation heads off buildup of ammonia by controlling relative humidity.

If any one of the above factors goes wrong for any length of time it means trouble. Fortunately, in most situations bringing in fresh air and exhausting toxic fumes are accomplished by ventilation aimed primarily at controlling temperature and moisture. Don't forget that the good in-house environment has to be distributed evenly throughout the house. Pockets of dead air, drafts, cold spots, or hot spots can lower flock performance and even cause mortalities.



### **How Ventilation Works**

In simplest terms, *ventilation* means moving outside air into the house, and exhausting inside air out of the house. Ventilation is a continuing, daily essential in poultry production throughout the year. However, winter and summer ventilation requirements are very different. Ventilation needs also change according to the age of the birds.

### Key Factors: Air Exchange Rate and Air Flow-Through Pattern

The amount of air your ventilation system needs to move into and out of the house depends on outside weather conditions and the age of the birds. Generally, the warmer it is and the larger the birds are, the more air the ventilation system needs to move. You need the proper *air exchange rate* for whatever conditions exist at the time. You might need to completely change house air on average once a minute or even less, or every five minutes or ten minutes, or longer. In a house using exhaust fan ventilation, the installed fan capacity in cubic feet per minute determines the maximum possible exchange rate; in a naturally ventilated house, outside wind conditions determine the maximum air exchange rate.

Conditions	Heat removal	Air exchange rate	Airflow pattern
1. Cool weather and/or young birds	No	Low	Bring air in high above birds for mix- ing; do not flow air directly onto birds (minimum ventilation).
2. Mild weather and/or larger birds	Moderate	Moderate (but higher than in Scenario 1)	Same as 1. Bring air in high for mixing; do not flow air directly onto birds ( <i>transitional ventilation</i> ).
3. Hot weather and/or large birds	Max	Max	Flow high-velocity air directly across birds for accelerated heat removal and wind-chill cooling ( <i>tunnel ventilation</i> ).

Also, the type of *airflow pattern* created in the house can make a critical difference. The most important decision is whether we need to protect the birds from chilling by keeping outside air from flowing directly onto the birds; or the flock needs the accelerated heat removal and wind-chill effect of flowing outside air directly onto them. The number, size, and placement of inlets; the incoming air velocity; the way outside and inside air are mixed; and the velocity and path of the air stream through the house all need to be matched to the needs of the birds.

Depending on the age or size of the birds and the outside air temperature, getting the right mix of air exchange rate and airflow pattern typically requires choosing one of three common ventilation scenarios:



# The Moving Target: Temperature For Best Bird Performance

At each stage of a bird's development, there is a fairly narrow "comfort zone" for optimum performance, where the bird can use the most of its feed energy for growth. If the temperature is too low, birds increase their feed intake but have to use more of that feed energy to keep their bodies warm. If temperature is too high, they reduce feed intake to limit heat production. The exact comfort zone temperature varies from flock to flock, depending on breed or bird genetics, bird sex, and feed formulation.

This optimum temperature zone is a moving target, changing as birds grow. Typically, a broiler growout starts around 90°F, with target temperature declining to near 70°F by week 6. The exact temperature profile will vary from flock to flock and from company to company. The chart below shows a generalized example optimum temperature regime.

**Important**: What matters is what is being experienced by the birds, not by the manager or by a thermometer mounted four feet above bird level. Thermostats and monitoring thermometers need to track temperature at bird level. Further, the temperature the birds experience will be very different from the thermometer reading during tunnel ventilation. The wind-chill effect of tunnel ventilation lowers the temperature experienced by the birds below the actual thermometer reading. When we are tunnel ventilating, "target temperature" means the equivalent temperature felt by birds. This is especially important to remember early in a growout. It can be disastrous to wind-chill stress young birds.

### **Basic Ventilation Systems & Setups**

Poultry house ventilation equipment can be as simple as manually-adjustable curtains over sidewall openings, or can include various sizes and types of computer-controlled fans and inlets. The first question to ask about how a ventilation system works is, How does it move outside air into and out of the house? And the most basic distinction to make is whether the ventilation system is fan-powered or relies on natural wind or air circulation.

**Natural Ventilation** relies on opening up the house to the right extent to allow outside breezes and inside convection currents to flow the right amount of air into and through the house. This is usually done by lowering (or raising) sidewall curtains, so it's most often called "curtain ventilation." Curtain ventilation as a system does not allow a great deal of control over in-house conditions.



**Fan-Powered Ventilation** uses fans to bring air into and through the house. Powered ventilation generally allows much more control over both the air exchange rate and the air flow-through pattern, depending on the configuration of fans and air inlets and the type of control used.

NOTE: Inside circulation or stirring fans often used in curtain-ventilation setups help with mixing of outside and inside air, preventing temperature stratification in cool weather, and to some extent cooling birds with direct breezes. This type of fan setup, however, does not move outside air into the house, so a curtain-ventilated house with stirring fans is not considered a *power-ventilated* house.

In the U.S. and worldwide, the number of houses using only natural (curtain) ventilation is declining, especially in warmer climates. However, many houses equipped with fan-powered ventilation systems also have curtain sidewalls and use natural ventilation when outside weather conditions are favorable.

### Principles of Natural (Curtain) Ventilation

Opening house curtains can quickly flow a large volume of outside air through the house, which essentially converts inside conditions to become the same as outside conditions. Curtain ventilation therefore is ideal when temperature outside is close to the temperature the birds need. The air exchange rate, however, will depend to a great extent on outside winds.



Curtain ventilation is best used *when outside temperature is the same as or not more than 10 to 15 degrees colder than you want the house to be.* The larger the birds, the larger the temperature difference can be because of the heat generated by the flock. When the outside temperature is lower than you want the house, a small curtain opening cycled on and off can maintain the temperature birds need and still give adequate air exchange.

The problem with curtain ventilation in cold weather is that with small curtain openings, heavy outside air comes in at low speed and drops immediately toward the floor, where it will chill the birds and cause moisture condensation, creating wet litter. At the same time, higher-level warmer air runs out of the house, resulting in large swings in in-house temperature and stress on the birds. If curtain ventilation is used in cooler weather, curtain machines operated on frequent on-off timers and with bird-level safety thermostats are essential. Circulation fans can help mix incoming cold and in-house warm air. *Curtain ventilation requires constant, 24-hour management*.

### **Types of Fan-Powered Ventilation Setups**

Fan-powered ventilation systems can use either positive or negative pressure. Positive-pressure wall-mounted fan systems, which push outside air into the house, are most often seen in setups used for cooler weather. However, most poultry house fan-powered systems now use negative-pressure ventilation. This means that the fans are exhaust fans, pulling air out of the house. This creates a partial vacuum (negative pressure) inside the house, so that outside air is drawn into the house through inlets in the house walls or under the eaves.



Achieving a partial vacuum inside the house during ventilation allows for much better control of the air flow-through pattern in the house and for more uniform conditions throughout the house. That is, both dead air areas and hot or cold spots are minimized.

Fan-powered, negative-pressure poultry house ventilation systems are most commonly seen in three major setup configurations:

- 1. <u>Minimum</u> ventilation (also called just "power ventilation" or even "power vent") used for cooler weather and/or smaller birds.
- 2. <u>Tunnel</u> ventilation used for warmer weather and/or larger birds.
- 3. <u>Transitional</u> ventilation used for "in-between" conditions.

All three of these ventilation setups use the negative pressure principal, but operate at different static pressures. Static pressure, in the U.S. measured in inches of water column, indicates the difference between in-house and outside air pressure, or the degree of partial vacuum achieved in the house.

Minimum-ventilation setups typically operate at static pressure ranging from 0.08 to 0.12 inches. Tunnel ventilation may produce static pressures ranging from 0.04 to 0.15 inches, measured at the center of the house, depending on design wind speed (higher wind speeds imposing higher static pressure) and the type of evaporative cooling system installed.

Important distinctions sometimes get overlooked in the way we talk about houses. We'll talk about a "tunnel house," for example, as though there was only one kind or mode of ventilation used. The tunnel setup is used only in warm to hot weather, and the "tunnel house" for cool weather or small birds is probably equipped for and switched either to sidewall exhaust fans and air inlets (minimum ventilation mode) or uses the sidewall inlets while running some of the tunnel fans (transitional ventilation mode). The changing needs of birds as they grow and the variability of weather, especially in fall and spring, requires growers to be ready to switch their ventilation system from one mode or setup to another when needed.



### **How Minimum Ventilation Works**

The purpose of the minimum ventilation setup is to bring in just enough fresh air to exhaust excess moisture and ammonia fumes during cold-weather conditions and/or when birds are very small. And, to do this without chilling the birds. Typically, from two to six 36-inch exhaust fans on one sidewall and horizontal inlets high on both sidewalls are used in this setup. The key to successful minimum ventilation is creating the proper partial vacuum (that is, static pressure) so that air comes in with sufficient speed and *at the same speed* through all inlets. With air inlets distributed evenly along the whole length of the house, air flow is then uniform throughout the house.



To get this needed air flow-through pattern in a minimum ventilation setup, the air inlet area must be matched to the fan capacity being used. <u>If the air inlet area is too small</u> (for the number of fans running), fans will have to work against too-high static pressure and will not deliver the air exchange rate needed. <u>If air inlets are opened too wide</u>, static pressure drops too low, and air will come in mostly or only through inlets closest to the fans, creating non-uniform air flow and poor conditions for birds. Using manufactured inlets actuated by a static pressure controller makes the inlet area adjustment automatic. Curtain cracks and fixed board inlets are not good inlets, allowing too-wide openings and dumping cool incoming air onto the birds. Minimum ventilation also requires a tight house: air leaks will tend to spoil the desired high-velocity air flow through the planned inlets.

Minimum ventilation is timer-driven, and may be set to operate as little as one minute in five early in a growout or in very cold weather. As birds grow larger and/or weather warms, thermostats override the timer to provide an adequate ventilation rate. Important: In cold weather the need to remove moisture from the house means that some minimum ventilation rate must be maintained even when the thermostat doesn't call for ventilation and even if a small amount of house heat must be removed in the process.



High-velocity tunnel ventilation airflow removes heat and provides wind-chill cooling

### **How Tunnel Ventilation Works**

The goal of tunnel ventilation is to keep birds comfortable in warm to hot weather by using the cooling effect of high-velocity airflow. The tunnel setup is especially suited to warmer areas and where larger birds (5-8 pounds) are being grown. Tunnel systems are designed first to handle the expected need for heat removal, providing the air exchange rate needed to exhaust excess house heat in hot weather.

The tunnel setup also provides wind-chill cooling, moving air as in a wind- tunnel through the length of the house. A velocity of approximately 500 to 650 feet per minute is needed for most effective wind-chill cooling.



Air Velocity (ft/min) The wind-chill effect created by high-velocity air can reduce the effective temperature felt by

The wind-chill effect created by high-velocity air can reduce the effective temperature felt by fully-feathered birds by as much as 10-12 degrees F. The graph below shows estimated effective temperatures that result with different air velocities, for 4-week and 7-week birds.

As the illustration shows, caution must be used in tunnel ventilating with younger birds, since they experience greater wind-chill effect for a given air velocity. Note that the "effective" temperature can only be estimated, not read from a thermometer or calculated. Bird behavior must be the guide to judging the right number of fans to turn on to create the air velocity and air exchange rate needed to keep birds comfortable.

The high-velocity airflow of the tunnel setup makes it well suited to adding evaporative cooling. This can be done either with in-house foggers or with evaporative cooling pads placed outside the air inlets. This real cooling of incoming air, on top of the "effective" cooling produced by wind-chill, can keep birds performing well even in very hot weather. Used alone, the wind-chill effect of tunnel ventilation becomes less pronounced as air temperatures rise much above 90°F, and above 100°F the air begins to warm instead of cool the birds.

### How Transitional Ventilation Works

The transitional ventilation setup is a hybrid system that enables a grower to provide effective negative-pressure ventilation in conditions where neither minimum nor tunnel ventilation would



be advisable. The transitional mode allows more precise control of the in-house environment during "in-between" moderate weather or in the growout transition time between the need for minimum ventilation and the need for high-volume air movement to get rid of heat build-up in the house.

Using the transitional setup can enable a producer to eliminate curtain (natural) ventilation altogether, using negative pressure powered ventilation year-round for better environmental control and better bird performance.



Transitional ventilation works by using some of the tunnel fans, either alone or in combination with sidewall fans, to bring air into the house through perimeter air inlets instead of through the tunnel inlets, which are kept closed. Outside air enters and mixes with in-house air. The big difference over the minimum ventilation setup is that the in-creased fan capacity gives a larger volume of air exchange. Running four tunnel fans in the transitional setup, for example, gives the same ventilation rate as running four-fan tunnel ventilation, but with no wind directly on the birds.

As with minimum ventilation, the air inlet area must be matched to the fan capacity used. In general, enough sidewall inlet area should be provided to operate at least 40-50% of the installed tunnel fans in the transitional mode without creating excessive static pressure. For most efficient operation, perimeter inlets are controlled by actuators, which control air inlet openings based on static pressure, as in minimum ventilation.

### How Evaporative Cooling Works

The simplest application of EC for broilers is the use of fogging nozzles mounted overhead in curtain-ventilated houses. The most efficient and effective modern systems, however, are designed to work in conjunction with tunnel ventilation. By adding some actual temperature reduction on top of the wind-chill cooling effect of tunnel, properly designed and operated EC systems can keep birds performing well in very hot weather.

The two major setup choices for tunnel-house EC are in-house foggers and wetted pads mounted over the tunnel air inlets. Foggers are very difficult to manage without wetting the birds or

floor. High-efficiency recirculating pad systems demand less management attention and do not risk wetting birds or litter. The predominant practice in severely hot climates is to install well designed 6-inch recirculating evaporative cooling systems and in addition place a small number (50-70) of 1 gal/hr fogging nozzles in the mid-house area. These nozzles are only used on the very hottest days to aid in reducing air temperature.

Starting air tem-	System	Resulting air temperature (°F) for given relative humidity				
perature (°F)	efficiency	40% RH	50% RH	60% RH		
100	50%	90	92	94		
	75%	84	87	90		
95	50%	85	87	89		
	75%	80	83	85		
90	50%	81	83	84		
	75%	76	79	81		

#### EVAPORATIVE COOLING POSSIBLE UNDER DIFFERENT CONDITIONS

The table below shows the in-house air temperatures that result given higher or lower starting air temperature, system efficiencies, and relative humidity. For example, if it is 95°F outside at 50% relative humidity, a 75% efficient EC system will give 12 degrees of cooling, to 83°F. If the tunnel wind-chill gives another 10-12 degrees of effective cooling, fully-feathered birds will feel like they are in 71°-73°F air.

EC can provide useful cooling even in areas usually considered quite humid. In the U.S. Southeast, for example, RH may reach 90% during a summer night, but typically drops to 50% or even lower by midday. A rule of thumb is that EC is very practical if there is at least an average 20-degree F difference between nighttime low temperatures and daytime highs.

### Making Good Ventilation, Housing and Equipment Decisions

Generally in the U.S. and increasingly throughout the world, improvements in ventilation technology have yielded increases in production efficiency which in turn are rewarded by increased income. The wisdom of particular ventilation choices, however, always depends on the particular situation and conditions. The most important factors in the decision process are usually prevailing weather, type, size and number of birds to be produced, cost and returns calculations for given equipment (including long- vs short-term cash and financing considerations), and the management and labor requirements imposed.

Unless weather is consistently in the cool-to-mild range throughout the year, growers in almost any situation find that it pays to upgrade from purely natural (curtain) ventilation to powered ventilation. In cooler climates such as the U.S. upper Midwest, only a minimum ventilation setup with sidewall exhaust fans and perimeter inlets might be needed. In warmer climates and especially when larger birds are being grown, tunnel ventilation and evaporative cooling have been found to give a profitable bird performance advantage.

In many poultry producing areas a large percentage of houses are now totally solid-walled, without any curtains Theses houses can be made extremely tight and therefore the environment in the house can be 100% controlled by the ventilation and heating systems These solid wall houses save fuel and can provide maximum comfort to the birds in hot and cold weather, thus



contributing to better flock performance. They must have highly reliable, well designed standby generators to prevent catastrophic losses in the event of utility power failure.

### **Benefits of Environmental Control**

Broiler production is meat production, and birds most efficiently convert feed to meat when they are given consistently optimum environmental conditions. Temperature is the most critical factor. Small temperature differences can have a significant effect on returns to the grower. This has been well confirmed by research and by experience under U.S. conditions.

The next several charts show actually monitored temperature variations recorded by "data logging" monitors in curtain-ventilated vs environmentally controlled broiler houses in the U.S. Southeast in autumn. While the conventional house allows very little if any temperature control, the environmentally controlled house tracks the daily target temperature (shaded line) fairly closely. That these results were recorded for the first 28 days of a growout is particularly significant.

The potential of the modern tunnel house to deliver better bird performance is enormous, as demonstrated by recent USDA research. Charts below show weight gain and feed efficiency improvements that can be realized with higher windspeeds under controlled conditions. For later stages of a growout and for warmer weather, especially when birds are grown to larger sizes (5-8 pounds), tunnel ventilation with evaporative cooling has been shown to give a definite performance advantage.

The table below shows actual in-field data recorded by a commercial broiler operation during summer growouts in the Southeast U.S., comparing conventional and tunnel ventilated houses with evaporative cooling. It must be stressed that the payoff from investment in ventilation technology can be realized only when systems are properly designed for the purpose and setting, with careful attention given to choosing component parts, and – equally important – are managed properly.



### Hot-Weather Performance: Tunnel + EC vs Conventional Ventilation

58-day broilers	Broiler weight (lbs)	Feed conversion	% Liva- bility	% Condem- nations	Live cost (cents/lb)
Tunnel + EC	7.2	2.18	92.4	1.71	21.8
Conventional	6.85	2.24	88.1	1.90	22.5

### **Conclusion/Summary**

It is imperative that every manager and poultry grower understands the basic principles of ventilation and then is able to apply those principles and concepts to their particular farming operation. Good ventilation, just like good feed, water, lights and husbandry, play an enormous role in production efficiency and profitability. More information on ventilation and housing is available at the National Poultry Technology Center website: www.poultryhouse.com.



# O<sup>12</sup> Epigenetic Adaptation of Broilers to Inside Temperature of Broiler House

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Climatic environment is one of the main limiting factors of broiler production. Because selective breeding for rapid growth rate increased susceptibility to environmental temperatures of today's commercial broilers, to keep the target temperatures for best broiler performance is a significant issue in broiler industry.

Epigenetics can be described as modifications of the phenotype without changes of the DNA sequence that are transmitted to the next generations. An example of epigenetic thermal adaptation is thermal conditioning of embryos during critical developmental phases that affects gene expression and improves thermal adaptation of broilers during post hatch.

This paper aims to review our studies on conditioning of broiler embryos using cyclic higher and lower incubation temperatures between 10 and 18 d of incubation on thermal adaptation of broilers.

# O<sup>13</sup> Effect of Flock Age (27 Wk: Young; 37 Wk: Prime) Hatch Time and Litter Temperature on Broiler Performance

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#### Abstract

This experiment was conducted to determine the effect of flock age (27 wk: young; 37 wk: prime) hatch time and litter temperature from 0 to 2d. (normal: 33-31°C, and cool: 26-28°C) on broiler performance. A total of 546 chicks, which were determined hatch time, were used in this experiment. Chicks from young flock had a lower feed consumption and BW at 35d than prime flock (P<0.05). Although cool brooding temperature reduced feed consumption at first 2d and BW at 7d (P<0.05), there was no significant effects on BW and feed consumption at 35d (P>0.05). Mortality at 35d was affected by flock age X litter temperature and hatch time X litter temperature. Young flock and late hatched chicks exhibited greater mortality on cool litter temperature condition (P<0.05).

**Keywords:** *litter temperature, broiler breeder flock age, hatch time, body weight, feed consumption, mortality.* 



### O<sup>14</sup> Ribotyping as a Tool for Epidemiological Typing of Salmonella Infantis in Broiler Production

### Özlem Şahan, K.Serdar Diker, Mehmet Akan

Ankara University, Faculty of Veterinary Medicine, Ankara, Turkey

### Abstract

The study aimed to illuminate the molecular epidemiology of *Salmonella* Infantis that isolated from a set of broiler farms located in a particular region. In this direction poulty litter and dust collected from 6 different broiler farms and total of 176 isolates of *S*. Infantis were used. Isolates were treated with restriction endonuclease PvuII, to cut DNA into smaller fragments and the fragments were separated by gel electrophoresis by using Ribotyping method. Separated fragments were transferred from gel to membrane and 16S-23S rRNA operon-specific chemiluminescent substrate was subjected to hybridization with the help of labeled probe. After hybridization band profiles that were called as Ribopattern were used for epidemiological typing of isolates in comparison with a reference database. The number and relationship of bands obtained by ribotyping were analyzed with a similarity index by using Dice coefficient and unweighted pair group method with mathematical averaging (UPGMA) in GelCompar<sup>TM</sup> (Applied Maths, Ghent,

Belgium) software. The results showed that ribotyping method in showed that there is a set of similar strains were isolated from litter and dust samples in the same poultry farm thus were made comments about transmission of strains between intra or inter integrates. Strains similarity was revealed that belongs to similar and different integrated. In addition, it was revealed that strains belong to farm in small-scale region. This results in the framework the methods showed that it was capabled to do epidemiological distinction of strains.

Key words: Broiler, ribotyping, Salmonella Infantis

# O<sup>15</sup> Quality Control in Vaccine Production and EU Regulations

### Daniel Windhorst

Lohmann Animal Health, Cuxhaven, German

### Outline

- EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use
- Release specifications for AviPro SALMONELLA DUO
- Salmonella control and legislation in the EU
- Vaccination as the first method of control

# EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use





Comission Directive 91/412/EEC of 23 July 1991 laying down the principles and guidelines of good manufacturing practice for veterinary medicinal products

Part I -	Basic Requirements for Medicinal Products
Chapter 1	Quality Management (revision October 2005)
Chapter 2	Personnel
Chapter 3	Premise and Equipment
Chapter 4	Documentation
Chapter 5	Production
Chapter 6	Quality Control (revision October 2005)
Chapter 7	Contract Manufacture and Analysis
Chapter 8	Complaints and Product Recall (revision December 2005)
Chapter 9	Self Inspection

- EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use
- Chapter 1 Quality Management
- Principle:
- The holder of a Manufacturing Authorisation must manufacture medicinal products so as to ensure that they are fit for their intended use, comply with the requirements of the Marketing Authorisation and do not place patients at risk due to inadequate safety, quality or efficacy.



EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 1 Quality Management
- Principle:
- The attainment of this quality objective is the responsibility of senior management and requires the participation and commitment by staff in many different departments and at all levels within the company, by the company's suppliers and by the distributors.



EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 1 Quality Management
- Principle:
- To achieve the quality objective in a reliable manner there must be a comprehensively designed and correctly implemented system of Quality Assurance incorporating Good Manufacturing Practice and thus Quality Control.




EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 1 Quality Management
- Principle:
- Quality Control is that part of GMP which is concerned with sampling, specifications and testing, and with the organisation, documentation and release procedures which ensure that the necessary and relevant tests are actually carried out and that materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory.
- Test methods have to be validated.



EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 2 Personnel
- Principle:
- The establishment and maintenance of a satisfactory system of quality assurance and the correct manufacture of medicinal products relies upon people.
- The manufacturer should have an adequate number of personnel with the necessary qualifications and practical experience.



EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 3 Premisis and Equipment
- Principle:
- Premises and equipment must be located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimise the risk of errors and permit effective cleaning and maintenance in order to avoid crosscontamination, build up of dust or dirt and, in general, any adverse effect on the quality of products.



EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 5 Production
- Principle:
- Production operations must follow clearly defined procedures; they must comply with the principles of Good Manufacturing Practice (GMP) in order to obtain products of the requisite quality and be in accordance with the relevant manufacturing and marketing authorisations.





EU Guidelines to GMP (Good Manufacturing Practice) Medicinal Products for Human and Veterinary Use

- Chapter 6 Quality Control
- Principle:
- Quality Control is concerned with sampling, specifications and testing as well as the organisation, documentation and release procedures which ensure that the necessary and relevant tests are carried out, and that materials are not released for use, nor products released for sale or supply, until their quality has been judged satisfactory.





#### 3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI

## What is special about Salmonella ?







### EU baseline survey on the prevalence of *Salmonella* in laying flocks of *Gallus gallus*

- European Union agreed a programme for the reduction of
- *Salmonella* of public health significance in farm animals
- under Regulation EC No 2160/2003
- Scientific basis for setting of targets by EU
- First of several baseline surveys organize level
- •

#### Control & Differentiation

Approaches for vaccination

	Types of monitoring				
		= 7	□ 🜏		
	Phase	Self-monitoring	Official monitoring	Serovars	
Breeders Any flock > 250 birds	Rearing Production	<ul> <li>1 day</li> <li>4 weeks of age</li> <li>2 weeks before lay</li> <li>Every 2 weeks</li> </ul>	Onset of lay     Middle of lay     End of lay (4-8 weeks before)	Top 5	
		•			
Layers	Rearing	<ul><li>1 day</li><li>4 weeks before lay</li></ul>		Top 2	
Any flock	Production	<ul> <li>Every 15 weeks</li> </ul>	<ul> <li>End of lay (9 weeks before)</li> </ul>		



#### Salmonella Legislation in the EU

Reference to Commission Regulations in the European Union

- Commission Regulation 2160/2003/EC
- Breeding flocks positive for S. Enteritidis or S. Typhimurium must be slaughtered and their eggs destroyed
- Fresh table eggs should originate only from layer flocks subject to a national monitoring and control program.
- Eggs from flocks infected with a Salmonella serotype of zoonotic importance should be treated (not sold fresh)
- For fresh poultry meat to be put on the market Salmonella must be absent in 25g of meat

#### Commission Regulation 1177/2006

- No antimicrobials shall be used to control Salmonella
- Vaccination is obligatory in member states when S. Enteritidis flock contamination > 10%



### Reported outbreaks of foodborne Salmonella







### Vaccination as the first method of control

### Role of vaccination against Salmonella

In the frame of a holistic approach

- □ **Vaccination** is the major intervention tool to prevent *Salmonella* infections because:
  - Reduces organ colonization
  - Reduces egg contamination (shell or vertically)
  - Reduces carcass contamination
  - Reduces excretion in the environment
  - Reduces the number positive flocks
- Success of vaccination is influenced by many factors (e.g. health status of the bird, vaccination technique, synergism with other products).
- Vaccination should be accompanied by a bundle of operational and strategic measures steered by management of an integration.





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### Commission Regulation 1091/2005 /EC

- Live Salmonella vaccines should not be used if the manufacturer does not provide appropriate methods to distinguish bacteriologically wild-type strains of salmonella from vaccine strains
- Easy differentiation for AviPro Salmonella DUO:
- □ Bacteriological distinction allows a rapid differentiation from the field strains, which is achieved by means of an antibiotic sensitivity test.

		AVIPTO. SALVONELLA DUO		AVIPTO. SALVONELLA DUO	
		SE vaccine strain	SE Field strain	ST vaccine strain	ST Field strain
Erythromycin	15-30 µg/ml	Sensitive	Resistant	Sensitive	Resistant
Rifampicin	200 µg/ml	Resistant	Sensitive	Resistant	Sensitive
Streptomycin	200 µg/ml	Resistant	Sensitive		
Nalidixic acid	20 µg/ml			Resistant	Sensitive



Summary of Product Characteristics (SPC) Legal category: POM-V Marketing Authorisation number: VM: 16894/4009

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI



### History of human Salmonella infections (EU level)

## EFSA opinion on vaccination

- EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2010
- EFSA Journal 2012;10(3):2597
- "The results above indicate that the reduction of S. Enteritidis in laying hen flocks and of Salmonella spp. in table eggs is likely to have contributed to the decline of S. 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, driven by the economic consequences of egg restrictions and the requirement to heat treat eggs from positive flocks".



2.8.2006

EN

Official Journal of the European Union

L 212/3

#### COMMISSION REGULATION (EC) No 1177/2006

of 1 August 2006

implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry

(Text with EEA relevance)

Vaccination of poultry is regarded as a relevant measure to increase protection

contamination of eggs shall be applied to all layers in the MS with a prevalence >10%

Vaccination against SE reducing the shedding and

- (2) The EFSA concluded in its opinion on the use of waveless for the control of solenomals in positry, that vaccination of poolity is regarded as an additional measure to increase the restaurce of birds against salmondla copours and decrease the shudding.
- (10) The EFSA in its opinion also stated in particular that provided that the detection methods are able to differentiate the waveles strains from while strains, both currently available inactivated and the waveless can be safely used throughout the life of the birds, except during the withdrawal period before stugitter and, with regard to like waveless, in laying hers during production. Vitchnation of layers is considered useful at measure to reduce shedding and egg comminiation, when the purpose is to reduce high prevalences. Sciencelle extendities is the most important cause of outbreaks in humans by the constantytion of eggs.

 Live submonella vaccines shall not be used in the framework of national control programmes in laying bens during production unless thusadity of the tast has been domonstructed and they are authorized for such purpose in accordance with Directive 2001/82/8C.

3. Vaccination programmes against Salasseik currentility reducing the shedding and contamination of eggs, shall be applied all used during maring to all lyging heres at the latest from 1 January 2005 on in Member States as long as they did not democratized a preachase below 10% based on the works of the baseline audy in accordance with Anticle 1 of Commission Decision 2004/06/58C or based on the monitoring to islow up the Community target, set in accordance with Anticle 40) of Regulation (EC) No 2160/2003.

### Compulsory Vaccination against Salmonella

Selected Countries Worldwide

	Com	oulsory	Subsidy		
Country	Breeder	Layer	Breeder	Layer	
Belgium	No	No	Yes (60% of total cost)	Yes (60% of total cost)	
Baltic countries	No	No	No	No	
Spain	No	Yes (> 9%)	No	No	
France	No	No	No	No	
Portugal	No	No	No	No	
Italy	Yes (if previous flock is +)	Yes (if previous flock is +)	No	No	
Greece	SE; ST	SE	Yes	Yes	
Cyprus	SE; ST	SE	Yes	Yes	
Bulgaria	No	No	No	No	
NL	No	No	Yes	Yes (0,02€ per dose)	
United Kingdom	SE; ST	SE	No	NO	
Hungary	No	No	Yes (2 doses)	Yes (2 doses)	
CZ	No	Yes			
Poland	Yes	Yes	No	No	
Germany	SE; ST	SE; ST	Some Federal States	Some Federal States	
Skandinavia	Prohibited	Prohibited	No	No	
Brazil	Prohibited	Yes	No	No	

Regulatory Affairs, Lohmann Animal Health Data on file (2013)

### Monophasic Salmonella Typhimurium

- Increasing numbers of outbreaks of monophasic Salmonella Typhimurium strains are reported in several EU countries
- Commission Regulation (EU) No 517/2011 confirms that these strains shall be included in the Salmonella reduction targets as described in Regulation (EC) No 2160/2003
- A study at the University of Ghent demonstrated that vaccination with Salmonella Vac T strongly reduced shedding and organ colonization after infection with monophasic Salmonella Typhimurium

Oral administration of the Salmonella Typhimurium vaccine strain Nal2/Rif9/Rttto laying hens at day of hatch reduces shedding and caecal colonization of Salmonella 4,12:I:-, the monophasic variant of Salmonella Typhimurium Sofie Kilroy, Ruth Raspoet, Rosalie Devloo, Freddy Haesebrouck, Richard Ducatelle, and Filip Van Immerseel Poultry Science (2015)

### Take home message

- **Uvaccine production follows GMP guidelines in the EU**
- Salmonella has a prolonged survival in the environment and susceptible birds (non-vaccinated ones) are highly exposed to several sources of infection.
- □ It is not possible to eradicate Salmonella because the wide range of contamination sources: *Natural reservoirs* (e,g, poultry, pigs, cattle, pets), *Vectors* (e.g. rodents, flies, darkling beetles) and *other sources* (e.g. feed, raw materials, fomites)
- □ Vaccination in general is the major intervention tool to prevent Salmonella infections è significant decrease of Salmonella prevalence in EU-27. (Although the low prevalence, the scientific community strongly recommends to continue vaccination in order to avoid giving the infection the chance to rise again).





#### O<sup>16</sup> Recent Developments in GMOs and Challenges in Turkey

#### Selim Çetiner

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#### Abstract

The new millenium we are living is also called the age of life scineces because of the rapid discoveries in biological sciences. Genetically modified organisms are not only used in agricultural production but also in medicine, industry and other aspects of everyday life. While the use of GMOs in medicine and industry are accepted in general, the public reaction against the use of GMOs in agriculture is rather high. These reactions are usually ideological or emotional as each GMO is commercialized only after passing rigorous safety asseessment procedures based on scientific methods. While discussions are going on GMO safety, now with the development of new plant breeding techiques debate has been started on whether these new plants should be subjected to the same science based risk assessments. If so, this would block the public research and SEM scientists in developing new plants and commercialize them.

As we have witnessed so far, despite wide acceptance of GMOs in certain countries due to benefits for farmers and the environment, rejection of GMO cultivation in countries like Turkey has brought financial burden not only on farmers but also the whole of food production industry. The cost of Biosafety Law and its implementation has been estimated around 1 billion USD.

If the Biosafety Law and its imlementation would not been changed to comply with the international and EU biosafety legislation, the agricultural and food production sectors will be negatively affected and while GMO opponents will benefit the most.

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

### O<sup>17</sup> Energy Transformation of Organic Waste Material (Poultry Manure) to Different Suitable Energy Generation Technologies

Reinhard Enzenebner IBD Energy Systems Inc., İstanbul, Turkey



# POULTRY LITTER to ENERGY

- Biomass is an excellent source for renewable energy because: It is not limited by scarce resources
- It provides truly local energy, using local resources
- Biomass power plants have very low environmental impacts
- Biomass plants fit well with the local community
- Farmers gain access to a new market for their products
- Biomass power plants support rural infrastructure and employment







### POULTRY LITTER to ENERGY General

- 1. BASICS to Organic Waste Material
- 2. TECHNOLOGIES for conversion of organic waste to energy
- 3. LEGAL ISSUES and ROAD MAP for Project Implementation
- 4. IBD Enerji experiences for biomass projects

### POULTRY LITTER to ENERGY Basics



1 to/d Poultry manure , <20%m.c. in Gasif/Comb.Plant

800 kWh electricity

#### 3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI



## POULTRY LITTER to ENERGY Biogas Fermentation- Example 1,5MWel







#### POULTRY LITTER to ENERGY Biogas Fermentation-Process

#### 1. Pre-treatment with BIOaccelerator

It helps with the next steps and raise the biogas yield

#### 2. Sand removal

- It depends on the dry matter content of the chicken droppings
- Liquid system
- Sedimentation
- Hydro cyclone
- Solid system
- Counter flow air and cyclone





#### POULTRY LITTER to ENERGY Biogas Fermentation-Process

#### 3. Biogas power plant

#### Pre-Tank

- Input mixing with recirculation
- Water addition if necessary
- Sand removal
- Fermenter/Post-fermenter
- Fermenter with internal heating
- Mixer for optimal mixing
- High quality double membrane roof with biogas storage
- Sand removal





11

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#### **POULTRY LITTER to ENERGY Biogas Fermentation-Process**

- 4. Technology container
- 5. Separator
- 6. Stripping
- 7. Drying





#### **POULTRY LITTER to ENERGY Biogas Fermentation-Process**

#### 5. Separator

#### Liquid / solid separation

- The aim is to reduce the dry matter content • for the recirculation
- Lignin/salts reduction in the liquid product ٠

#### 6. Stripping

#### NitroStripp

- Ammoniac nitrogen stripping for recirculation
- Ammonium sulphate production •
- Utilization of the thermal energy produced by the CHP





13

12





### POULTRY LITTER to ENERGY Biogas Fermentation-Process

#### 7. Drying

#### BIOdry

- Digestate drying by means
- of the CHP heat
- Solid and liquid (ammonium
- sulfate) fertilizer production
- Digestate reduction

14



### **POULTRY LITTER to ENERGY** Biogas Fermentation - Assessment

- ADVANTAGES
- High electrical efficiency (30%-35%)
- High overall efficiency (70%-77%)
- Moderate CAPEX (3-4 mio €/MW)
- Low input material requirements for electricity generation (0,6 MWh/ton, broiler manure)
- Short construction period (10-12 months)
- Beneficial solution for smaller capacities (<5 MW)
- Fertilizer is produced as a side-product and has a market value
- Low OPEX (2 employee/shift)
- Gas motor (less maintenance cost)
- Continuous operation, short maintenance periods (op. time > 8300 hours)
- Waste water can be used as process water
- Hot water production from gas motor (<90° C)
- DISADVANTAGES
- For bigger power production capacities (>5MWel) several units have to be installed
- Input material must be collected carefully (no inorganic materials allowed)
- Used technology requires high amount of water consumption
- Liquid fertilizer needs to be disposed

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### POULTRY LITTER to ENERGY Biomass Gasification - Gasifier Schema





### POULTRY LITTER to ENERGY Biomass Gasification – Technology span

	Drying	Torrefaction (Roasting)	Devolatilization (Pyrolysis)	Gasification	Combustion
Temp. (°C)	80 - 140	- 140 - 350	-360 - 600	650 - 900	800 - 900
Process Oxygen	Low	0% O <sub>2</sub>	Sub stoichiometric $O_{\ell}$	Sub-steichiometric Q.	Excess O <sub>2</sub>
Volatiles remaining	100%	75% - 90%	0 - 18%	6%	ж
Fixed Carbon remaining	100% FC	100% FC	50 - 100% FC	0 17% FC	0% FC
Off-Gas	Water Vapour	Some CO, CO <sub>2</sub> Organic Adda	солооунусун,	00/00/H/C(H,	00,+11 <u>,</u> 0
Solida	Dry Freduct	<ul> <li>Reasteri preduct (smokeless fuel)</li> <li>Embridled &amp; hydrophosic</li> </ul>	<ul> <li>Char product</li> <li>Most word les driven off</li> <li>FC and ash remains</li> </ul>	<ul> <li>Ash product</li> <li>Low residuel FC</li> </ul>	<ul> <li>Ash product</li> </ul>
		17			





### POULTRY LITTER to ENERGY Biomass Gasification - General Layout

#### A general lay-out of a biomass combined heat and power gasification plant



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### POULTRY LITTER to ENERGY Biomass Gasification-realized 1MW Plant

#### GASIFIER

- The Gasification system, can be fired with different waste material, plastic, dirty wooden chips, dried manure, etc.
- The **downdraft gasifier prevents** the formation of bridges. **The drying** is needed to maintain 20-25% moist. level. To achieve better control and reaction mechanism, input material must be **pelletised**.
- Start up and operation of this technology in 2008, system upgraded in 2011 In its present configuration, it is capable of producing enough syngas to generate **up to 1,0 MWel** of electricity via a reciprocating gas engine.

#### GAS CLEAN UP

• This gas clean up has 6 stages to ensure that the inlet Syngas quality is suitable for running an gas engine.

#### WATER CLEAN UP

The **system cleans most of the water that is circulated around the wet scrubbing processes**. However, due to the moisture content in the inlet fuel it cannot be a totally closed system as the water condensed out of the syngas must be discharged.

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI



### POULTRY LITTER to ENERGY Biomass Gasification-realized 1MW Plant





### **POULTRY LITTER to ENERGY** Biomass Gasification - Assessment

#### ADVANTAGES

- Smooth Overall efficiency (60%-65%)
- Moderate CAPEX (3-4 mio €/MW)
- Construction period is approx. 11-12 months
- Simple technology, easy to handle
- ORC and Steam Turbine electricity generation unit easy to operate and maintain for capacities 0,5MWel...1,5 MWel
- Non-hazardeous ash, easy to dispose
- Excellent solution for smaller capacities when fertilizer is not required
- Flue gas cleaning equipment not needed (depending on the characteristics of input material)
- Low OPEX (2 employee/shift)
- Gas motor can be used for small capacities (<1,5 MW) as electricity generation unit
- Waste heat can be used for drying process of feedstock as well as for external use.

#### DISADVANTAGES

- Input material must have less than 20% moisture content (otherwise dryers required)
- For bigger capacities several units are required
- Low electrical efficiency (25%-35%), due to gas engine





### **POULTRY LITTER to ENERGY Biomass Combustion**



Broiler manure



Wood chips





**Fuel Spectrum** 







23

#### Ď٥ **POULTRY LITTER to ENERGY Biomass Combustion** Criteria for choice of technology Given fuel quality is the main criteria for the choice of combustion system Water content of 3 2 Lower Heating Ash content, fuel impurities, Particle size Value



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### POULTRY LITTER to ENERGY Biomass Combustion





### POULTRY LITTER to ENERGY Biomass Combustion - Grate Firing

#### **Boiler with Grate Firing**

- · Burning chamber integrated in boiler
- Natural circulation boiler with membrane walls
- Secondary air and recirculation gas above grate
- SNCR to reduce NOx emissions (lances)
- · Empty path and convective path
- Recirculation of fly ash
- "Biomass super heaters"
- Economizer in steel casing
- · Boiler cleaning with sootblowers
- S.H. steam temperature control with desuperheaters







### POULTRY LITTER to ENERGY Biomass Combustion - BFB

#### **Bubbling Fluidized Bed Firing (BFB)**





### POULTRY LITTER to ENERGY Biomass Combustion - Assessment

Fuel Quality					
	Grate Technology	BFB Technology	Reason	Economic Effect	
LHV max	15 MJ/kg	~20MJ/kg	wear on grate	maintenance cost	
LHV min	5.5 - 6 MJ/kg	3 - 4 MJ/kg	intensified heat transfer in BFB	choice of technology	
Co-combustion of wet fuel (LHV = 0)	low quantity possible	high quantity possible	intensified heat transfer in BFB	choice of technology	
Fuel grain size (screen size)	< 350 mm (max, pieces > 1m)	< 100 mm (sum of odge length « 300mm)	fuel feeding / desing	higher costs for fuel treatment with BFB	
Metal content	high content acceptable (depending on species), ramoval recommended	must be removed as far as possible (spec. Al, bulk particles)	agglomeration in BFB; blocking of deaching system	higher cost for fuel preparation with BFB	

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### POULTRY LITTER to ENERGY Biomass Combustion - Assessment





### POULTRY LITTER to ENERGY Biomass Combustion - Assessment

	Grate Technology	BFB Technology	Reason	Economic Effect
Cleaning intervals of soot blowers	1 – 3 cycles / day	0 – 1 cycle/ day	combustion     temperature     "self-cleaning effect"	<ul> <li>reduced wear from soot blowers</li> <li>HP steam consumption</li> </ul>
Ovartz sand demand	none	approx. 2 - 3kg/MWh	<ul> <li>efficiency of sand separation system</li> <li>sand attrition</li> </ul>	costs for sand acquisitio
Start-up burners	not required, sometimes executed	/equired	BFB requires bed material prohesting to the solid fuel ignition temperature	fuel costs, investment costs
Pressurized air	va	lable	- bag filter or ESP - SNCR - deashing system	costs for pressurized air production





# POULTRY LITTER to ENERGY Biomass Combustion - Assessment

	Grate Technology	BFB Technology		Reason	Economic Effect	
Slag (from grate), bed ash	high	low		BFB: bed ash consists of coarse particles only BFB: all other ash is boller / filter ash Grate: ash is only partially discharged with the flue gas; major fraction is slag from grate	bensiti of grate tiring since stag / bed ash is usually easier / cheape disposable compared to filter ash	
Boller / filter ash	low	high	1.2			
Wear	high (depending on fuel)	low	12 King	BFB: no moving parts Grate layer is a wear part which needs to be changed remained (5 - 10 years)	wear parts and maintenance costs	



### **POULTRY LITTER to ENERGY Biomass Combustion - Assessment**

	Grate Technology	BFB Technology	Reason	Economic Effect
Availability	up to 95% unavailability 	up to 98% unavailability «2001/a	no moving parts in furnace desching and fuol feeding systems can be executed redundantly	Runtime directly Influences the operating time / year
Continuous boller operating time	4000 - 6000h/a (depending on fuel)	4000 - 8500h/a (depending on fuel)	<ul> <li>reduced fouling in BFB</li> <li>Less mechanical wear (BFB)</li> </ul>	Runtime directly influences the operating time / year

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### **POULTRY LITTER to ENERGY** Biomass Combustion - Assessment

#### Summary

- · Main advantage of grate firing is low demand on fuel preparation
  - Fuel particle size
  - Inorganic impurities (ceramics, glass, Fe and non Fe)
  - Main advantages of bubbling fluidized bed are:
    - Availability
    - Emissions (usually no need for SNCR)
    - Efficiency
    - Wide LHV and water content range, high fine content in fuel possible
- Operational costs are similar
- Maintenance costs are lower for BFB
- Investment costs depend very much on the overall situation
  - Boilers including firing have very similar investment costs
  - In case extensive fuel preparation is required, grate technology is usually less expensive





### **POULTRY LITTER to ENERGY** Biomass Combustion - Comparison

COMPARISON OF TECHNOLOGY						
Те	chnologies	BIOGAS	GASIFICATION	COMBUSTION		
Parameters		Fermentation	Downdraft	fluidized bed		
Input material	% m.c.	manure > 70 %m.c.	manure < 25 %m.c.	manure < 30 % m.c.		
Output		biogas-electricity thermal heat	electricity thermal heat	electricity thermal heat		
Overall efficiency	%	70%-77%	60-65% 2)	85-90% 2)		
Electrical efficiency	%	35%-38%	25-35%	30-37%		
CAPEX	Mio€/MW	3,2-4,6	3,0-4,0	4,5-7,0		
<b>Construction period</b>	months	9-12	12-15	18-24		
OPEX per year	€/MW	medium 1)	medium 1)	higher 1)		
economic range for electrical capacity	MWel	1 < 5 MW	0,5 < 10 MW	> 5 MW		
Emissions acc.to EU-Standards	12	no significant add. investment needed	add. investment (<30%) needed 3)	no significant add. investment needed 4)		
Side product		Organic solid and liquid fertilizer	Non-hazardeous ash	Non-hazardeous ash usable as fertilizer		

legend: 1) depend on electricity generation

- 2) linked to input material
- 3) for gas engine only, not for ORC+turbine
- 4) for the presented technology only valid





### **POULTRY LITTER to ENERGY** Legal Issues - Electricity Law and Regulations

In Turkey, laws are continuously changed by the governmental authorities.

#### 2 types of generation:

- 1. Non-licensed power plants ( max. 1 MW)
- 2. Licensed power plants (>1 MW)
- Feed-in tariff is valid for 10 years, depending on the type of the renewable source-situation for chicken is not different.
- Incentives (for custom duties, value added tax rates, etc.) are available for procurement of energy generation equipment.
- Licensed generation is divided into 2 parts (pre-license and license) and you cannot get license unless you fulfil your requirements given by EMRA.
- If land acquisition will be done with expropriation, the procedure is a long lasting process and should be considered carefully.



### POULTRY LITTER to ENERGY Legal Issues – Feed In Tariff

Facility Type and Renewable Resource	Prices (\$ cent/kWh)
Hydroelectric	7,3
Wind	7,3
Geothermal	10,5
Biomass (Incl. Landfill gas)	13,3
Solar	13,3

All these are **base prices**, there are **bonus** possible if domestically manufactured equipment is used in the power plant

### 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI



### POULTRY LITTER to ENERGY Legal Issues – Feed In Tariff

RESOURCE	ТҮРЕ	PART	\$ cent/kWh
		1- PV Panel integration and solar mechanical parts	0,8
		2-PV Moduls	1,3
	PV Plants	3-Solar Cells	3,5
		4-Inverter	0,6
		5-Solar Concentration Lens (GLASS)	0,5
SOLAR		1- Radiation Collection Tubes	2,4
ENERGY		2-Reflective Plates and Surfaces	0,6
CSP	S.A.S.	3-Solar Tracking System	0,6
	CSP	4- Mechanical components of solar thermal storage system	1,3
	$\wedge \wedge \wedge$	5- Steam Generation mechanical components	2,4
		6-Stirling engine	1,3
		7-Mirror integration and solar mechanical parts	0,6
Biomass		1- Fluidised Bed Boiler	0,8
Power		2-Liquid and gas fueled boilers	0,4
Plants		3-Gasification and gas cleaning group	0,6
(Incl.	Biomass	4-Steam or gas turbine	2
and		5-Internal combustion engine or stirling engine	0,9
landfill		6-Generator and power electronics	0,5
gas)		7-Cogeneration system	0,4



#### POULTRY LITTER to ENERGY Legal Issues – Feed In Tariff

- At least % 55 of the all equipment must be manufactured in Turkey to claim for bonus.
- Manufacturer must get **relevant permit and license** in order to manufacture goods in complience with the regulations.
- Typical formula for calculation of net electricity export price;
   Base Price + (percentage of local components x bonus rate)
- Relevant income tax and similar rates will be impended by government while you are exporting electricity to grid.
- Duration of feed-in tariff is **10 years** after acceptance from EPDK is completed.





### **POULTRY LITTER to ENERGY**

Legal Issues – Non Licensed Energy Generation

Cases which do not require a generation license:

- All generation plants with an installed capacity <1 MW (gross capacity)</li>
- 2. Power plants which utilizes for energy generation municipal waste or treatment of municipal waste materials
- 3. If **produced energy** will be used **to cover the internal demand** without any export to grid no limit for installed capacity given!



#### POULTRY LITTER to ENERGY al Issues – Non Licensed Energy Generation

Documents and permits for the project approval by government:

- 1. Call for interconnection agreement and view letters from grid operator
- 2. For Solar Plants: Approval letter for wind-snow load calculations by municipality (or city council).
- 3. Availability of **plant site area approval** by municipality (or city council).
- 4. System Basic Information Questionnaire (tech. specification of equipment)
- 5. System designer ID (declaration of legal identity)
- 6. Other relevant documents according to the law-regulation
- 7. Land site ownership or rental documents.

### 3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI



### **POULTRY LITTER to ENERGY**

Legal Issues – Non Licensed Energy Generation

#### Calculations for the project approval by government:

- 1. Energy losses, fall of potential, cable selection and dimensions
- 2. Equipment selection, max-min. Inverter calculations
- 3. Short circuit, AC-DC cable calc.
- 4. Internal demand transformer power calculations
- 5. Compensation equipment
- 6. Lighting arrester and earthing,
- 7. Lighting

## Beside these documents, there are other documents which must be given throughout the applications process.

These are all related project plots, sheets, etc.



### POULTRY LITTER to ENERGY Legal Issues – Licensed Energy Generation

#### For all projects >1 MW 2 phases for licensing required:

- Pre-license (simplified generation license)
   Duration of Pre-license >24...<36 months depend on the project.</li>
- License period to get electricity generation license After getting the pre-license following requirements have to be fulfilled:
  - 1. Land acquisition (or expropriation),
  - 2. Order of energy generation equipment,
  - 3. Project engineering works,
  - 4. Approval of zoning plans,
  - 5. Approval of pre-project (by EPDK and governmental bodies)
  - 6. EIA decision
  - 7. Application for interconnection agreement to TEIAS

**Electricity generation license** will be granted, when all these requirements are fulfilled.

License duration is not fixed by legistlation, it depends on declaration in project plans and forms and approval of EPDK.





### POULTRY LITTER to ENERGY Legal Issues – Licensed Energy Generation

Calculations for the project approval of renewable energy plants to government:

- 1. Energy losses, fall of potential, cable selection and dimensions
- 2. Equipment selection, max-min. İnverter calculations
- 3. Short circuit, AC-DC cable calc.
- 4. Internal demand transformer power calculations
- 5. Compensation equipment
- 6. Lighting arrester and earthing,
- 7. Lighting

Beside these documents, there are other documents which must be given throughout the applications process.

These are all related project plots, sheets, etc.



### POULTRY LITTER to ENERGY Road Map – Project Realization

Duration for **project realization till commercial operation** (after contract signing and downpayment) for:

- Biogas Fermentation Plant: 9...12 months
- Biomass Gasification Plant: 12...15 months
- Biomass Combustion Plant: 18...24 months

### O<sup>18</sup> Challenges and Opportunities in Evaluation of Poultry Litter as Fertilizer

#### <u>Mustafa Kaplan</u>, Ahmet Şafak Maltaş

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#### Abstract

In this study, to be encountered approximately 5.5 million tons of poultry waste is seen as the environmental problem when used as input in crop production, some ordinary problems and the measures to be taken against these problems are evaluated. Every season of the year to constantly emerging waste, environmental and economic solution to create techniques and technologies should be developed. However, there is a need for the product to be used as effectively experienced and knowledgeable people.

Key words: Chicken manure, organic manure, salinity, composting



#### O<sup>19</sup> Effects of Fresh and Composted Poultry Litter on Soil Aggregations

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#### Abstract

The annual poultry meat production is about 1,8 million tons in Turkey, and produced broiler manure is nearly 2,1 million tons associated with meat production. This increase in broiler manure production leads to some environmental concerns. For this reason, poultry enterprises sometimes encounter questions with society, municipalities and government institutions. In fact, broiler manures should be used in agricultural production on account of having high amount organic matter and plant nutrients contents. At first fresh broiler manures were mixed at the rate of 0%, 1%, 2%, 3% and 4% with soil and their mixtures were incubated for 45 days. Organic carbon, water stable aggregates, hydraulic conductivity, aeration porosity and available water content were determined in samples. Both manures increased investigated characteristics due to the fact that their high organic carbon contents except available water content. Fresh and composted manures did not cause difference for water stable aggregates. Although, the effect of both manure doses on water stable aggregates were statistically significant. As manure doses were compared each other, it was observed that 3% and 4% doses were more significant than others.

#### O<sup>20</sup> Presence and Biocontrol of Listeria Monocytogenes in Turkey Meat

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#### Summary

Poultry are playing a significant role in human foodborne infections because they are frequent vehicles of some human pathogens, which can spread in a short time to all the animals and are associated with cross contamination during production and processing. During stable to table, in order not to take hygienic precautions, contaminations with pathogenic microorganisms such as *Listeria* spp. may be occured and consumption of such turkey meat and meat products can cause foodborne illnesses. L. monoctogenes is a zoonotic food-borne bacteria that leads to a variety serious infections in humans such as encephalitis, meningitis, abortion and septicemia, and those suffering with listeriosis occurs in approximately 30% mortality. Epidemiologic studies have revealed that a significant proportion of cases of listeriosis caused by contaminated foods. The pathogen is widely distributed in the environment and well adapted to very different environmental conditions like tolerating wide temperature (0-45°C) and pH ranges (pH 4,3 - 9.6) make it difficult to control food-borne infections. Determination of the potential risk factors and development of strategies to combat with L. monocytogenes are only possible with a good characterization. L. monocytogenes serotype determination is a widely used method for the characterization which is important for epidemiological studies. Although there are 13 known serotypes of L. monocytogenes, according to epidemiological studies, approximately 95% of the isolates from the food and 98% of the clinical isolates that isolated from cases of listeriosis in humans belong to 1/2a, 1/2b, 1/2c and 4b serotypes. Consumption of turkey meat is increasing in Turkey. Related with the production technology, cross contamination risk is very high during processing, so it is important to control L. monocytogenes in turkey meat. Bacteriophages can be applied to living tissues without causing any harm due to their highly selective toxicity. This is the most important advantage when they compared with antibiotics and antiseptics. Rapidly growing bacterial resistance to antibiotics and need for development of alternative methods, increasing interest in using bacteriophages in treatment or as biocontrol agents in foods nowadays. In addition to the systems like HACCP and GMP for food safety from farm to table, the use of specific virulent bacteriophages for L. monocytogenes in order to reduce L. monocytogenes load in foods of animal origin before, during and after slaughter processes emerges as an another method. It is reported that the usage of specific virulent bacteriophages to L. monocytogenes as a biocontrol and decontamination agent of L. monocytogenes in foods, don't cause any side effects in humans.



#### O<sup>21</sup> Class 1 Integrons in Multiple Antibiotic Resistant Salmonella Infantis Strains

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#### Abstract

Relative prevalence of Salmonella Infantis to other Salmonella serotypes in poultry production has increased in recent years. With this increase, it is important to know the antimicrobial resistance patterns and mechanisms of *Salmonella* Infantis strains as seen in other Salmonella serotypes. In this study, genes related with multiple antibiotic resistance in Salmonella Infantis strains and genetic organization of these genes were investigated. The susceptibility of Salmonella Infantis strains to 12 antibiotics from 6 groups (beta-lactam, quinolone, chloramphenicol, aminoglycoside, tetracycline, sulphonamide) were examined by disc diffusion method. Integrase gene (*int*1) for determining class I integron and the presence of kanamycin (aphA1) and sulphonamide (sul1) resistance genes were analyzed by PCR. Multiple antibiotic resistance (4 to more antibiotics) was found in 91% of 150 Salmonella Infantis strains from poultry. Class I integron was observed in all multiple antibiotic resistant strains. Resistance associated aphA1, sull genes were found in all kanamycin and sulphonamide resistant strains. Sulphonamide and kanamycine resistance was not determined in 56% and 18.6% of integron carrying strains, respectively. High level of homology was observed according to DNA sequence analysis of integrase gene of Salmonella Infantis strains. In conclusion the presence of class I integron is almost all strains of Salmonella Infantis as compared with other Salmonella serotypes and Gram negative bacteria, showed the potential importance of these strains as recipient for antibiotic resistance.

#### O<sup>22</sup> Human Salmonellosis Attributable to Poultry: Disconnect between Science, Policy, Regulation, and the Public's Expectations

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#### Introduction

*Salmonella* continues to be a significant public health concern in the U.S., and although the prevalence of *Salmonella* has decreased in many meat products, salmonellosis rates in the U.S. have remained steady since 2003. Based on source attribution analyses, half of the reported sporadic *Salmonella* infections in the U.S. are attributed to poultry consumption (1), and several recent multistate outbreaks of salmonellosis in the U.S. have been linked to poultry. These observations raise two fundamental questions. First, is the current inspection of poultry by USDA-FSIS a sufficient public health indicator such that changes in *Salmonella* prevalence provide an accurate prediction of changes in salmonellosis burden? Second, does the current foodborne illness outbreak definition consistently protect public health?

#### Managing Salmonella in the poultry production system

Most poultry production companies manage *Salmonella* in their systems based on "regulatory risk." In other words, companies have *Salmonella* targets that are based on levels of *Salmonella* allowed in different products during inspection. Unfortunately, the way in which poultry is inspected in the U.S. and the *Salmonella* performance standards that are tied to poultry production have limited ability to predict human health outcomes. It is routinely stated by USDA-FSIS that reductions in the prevalence of contaminated poultry will result in decreases in illness attributable to poultry (2), but this is only true if prevalence, as measured, is an accurate measure of probable human exposure.

*Salmonella* prevalence and microbial loads can spike in a narrow window of time, for instance over several flocks or production days, and then be undetectable for an extended period (3). A company could pass its regulatory performance standard testing and still be putting a significant amount of product into commerce that can make people ill. If the desire is to reduce the likelihood of having product linked to human illness, companies need to start managing to a "public health risk" rather than a "regulatory risk." This entails having an understanding of *Salmonella* within the entire system and reducing the amount of finished product that is shipped into commerce that contains levels of *Salmonella* capable of causing disease.

#### Salmonella outbreaks attributable to poultry consumption

The way in which *Salmonella* outbreaks are defined does not appear to distinguish between ready-to-eat and not-ready-to-eat meats, even though the presence of *Salmonella* in each has completely different regulatory implications, risks and ramifications. Current outbreak definitions are also hampered by variability in surveillance systems, regional capabilities and market structure, and a fundamental gap between public health expectations and inspection strategy. Specific characteristics of the pattern of illnesses that also play a role in determining the


outbreak include: 1) Frequency, whether the number of observed illnesses exceeds the number that would be expected; 2) Attribution, whether a specific bacterial strain from a human illness can be attributed to a specific food commodity and to a specific source of the food; 3) Virulence, whether the apparent severity of the illnesses, usually reported as the frequency of hospitalization or the frequency of invasive disease, such as bloodstream infections, seems excessive; and 4) Antibiotic Resistance, whether the bacterial strain(s) associated with an outbreak possess antibiotic resistance traits (4). Unfortunately these criteria have been applied in a very subjective manner and do not differentiate RTE from NRTE sources.

#### Salmonella infection in the bird

Effective *Salmonella* control requires an understanding of the dynamics of pre-harvest *Salmonella* contamination and colonization of the bird. To effectively understand the dynamics of *Salmonella* occurrence on farm, it is necessary to realize that serotype and even strain differences contribute to the propensity of *Salmonella* to colonize and persist within the bird. Currently there is concern that the *Salmonella* that is present in the finished product has less to do with surface contamination and is instead related to the ability of certain strains to systemically colonize the bird. In creating a comminuted poultry product such as ground turkey, these *Salmonella* that were previously sequestered in systemic tissues can be released and contaminate the final ground turkey product.

It is well established that certain *Salmonella* serotypes have greater propensity than others to establish systemic infection in the avian host. For example, many studies have demonstrated the ability of certain *Salmonella* serotypes possessing human pathogenic potential to systemically infect poultry, including *S*. Enteritidis (5,6), *S*. Typhimurium (6,7), *S*. Heidelberg (6), and even *S*. Kentucky (6). Overall, we know very little about the nature of *Salmonella* colonization and persistence in commercial poultry and how this correlates with the presence of *Salmonella* on the meat product

#### Systems approach to Salmonella control

We must recognize that food safety is a continuum, and the final point of intervention is the consumer. This point is one over which agencies and industry have little control, however, and therefore we must target efficacious and cost-effective interventions before this point. We cannot utilize the "just cook it" mantra as a means towards improved food safety, but we also need to be realistic with illnesses attributable to food products of varied and variable risk. Zero tolerance of bacteria in NRTE meat is not a realistic near-term answer to this problem. Instead, understanding the variability in contamination levels and temporal clustering of contaminated product, and then developing sampling strategies to monitor contamination that directly predict human health outcomes is a starting point to improving the safety of NRTE meat.

To make strides at reducing human salmonellosis attributed to poultry, producers must begin to understand the dynamics of *Salmonella* within the entire system. Emphasis must be places on the breeder flocks. Contamination in the breeder flocks can lead to constant *Salmonella* introduction to the growout flocks. Once the breeder flocks are clean, systemwide interventions in the growout flocks will have greater efficacy. Companies need to place greater expectations on the primary breeder companies to supply replacements that are *Salmonella*-free, but this expectation will only be effective once interventions are successful in the breeder flocks. Finally, an understanding of *Salmonella* dynamics within the system will enable the development of specific management

practices that can help the processing plant account for variable levels of *Salmonella* being brought to the plant with the incoming birds. A systems approach to understanding *Salmonella* dynamics can aid in this process.

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# O<sup>23</sup> Future Perspective of Poultry Nutrition

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#### Abstract

It is obvious that many new trends and alterations are waiting for us in poultry industry under the economical, social and biological trends. Efforts for the evaluation of feeds that broilers' consume, studies on improving animal health and performance arise with positive outcomes. Because of feed cost represents over 70% of live production cost of poultry, to meet optimum nutrient requirement and to formulate optimum ration have a great importance. Then, functional amino acids and net energy systems are considered on poultry ration formulations.

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# O<sup>24</sup> Effects of Prebiotics, Probiotics and Essential Oils in Helping to Support Anticoccidial Activity

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#### Abstract

Coccidiosis is a disease caused by protozoan parasites of the genus *Eimeria* that can seriously affect the health and productivity of livestock, particularly in modern, intensive production systems. It results in a decline in general health, a failing appetite and eventually a loss of condition, and under severe conditions, even death. Infected livestock are also more susceptible to other diseases. From an economic perspective, coccidiosis is considered one of the top three costly pathologies in poultry with annual global economic losses estimated at over 2.0 billion dollars annually. To prevent livestock suffering and to limit economic losses, anticoccidial feed additives have been and continue to be routinely and successfully used. However, the emergence of drug-resistant strains of *Eimeria*, consumer concerns over in-feed prophylactic drug inclusion, and the anticipated withdrawal of coccidiostats at the end of the 2012 means alternative methods of controlling coccidiosis need to be considered. Vaccines are being developed and alternative feed additives based on bioactive compounds found in plants are currently being investigated.

The favourable fore heading methods of replacing anticoccidial feed additives are vaccines and botanical preparations in currency. Essential oils of some aromatic plants with substantial antiprotozoal potentials offers considerable alternative to synthetic anticoccidial compounds. Moreover, mannanoligosaccharides and lactobacillus based probiotics are also regarded as the promising alternatives to anticoccidials.

Keywords: Poultry, coccidiosis, anticoccidial feed additives.



# O<sup>25</sup> Effects of Herbal Vitamin D<sub>3</sub> Supplemantation and Calcium, Phosphorus Level on Growth Performance and Bone Development of Broilers

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#### Abstract

A trial was conducted to assess the effects of Panbonis (a herbal vitamin  $D_3$  source) on performance, some carcass characteristics and tibia parameters of broiler chickens. Mixed-sex 11200 day old Ross × 308 broilers were distributed into 7 dietary treatments with 8 replicates. Pelleted feeds based on corn, soybean and wheat were given adlibitum. Herbal Vit  $D_3$  were supplemented 0.01 and 0.02% with the presence of phytase into diets including decreased level of Vit  $D_3$  from commercial 5000 to 3000 IU. So dietary treatments were consisted of 7 groups as T1 (5000 IU Vit  $D_3$  at standart Ca and P level); T2 (5000 IU Vit  $D_3$  plus Phytase at standart Ca and P level); T3 (3000 IU Vit  $D_3$  plus 0.01% herbal Vit  $D_3$  plus Phytase at standart Ca and P level), T4 (3000 IU Vit  $D_3$  plus 0.02% herbal Vit  $D_3$  plus Phytase at standart Ca and P level), T5 (5000 IU Vit  $D_3$  plus Phytase at low Ca and P level), T6 (3000 IU Vit  $D_3$  plus 0.01% herbal Vit  $D_3$  plus 0.02% herbal Vit  $D_3$  plus 0.0

At 24th days of age 2 chicks from each replicates near to average weight of the pen were selected and killed for bone measurements. All birds were weighed and 2 of those slaughtered for evaluation of performance and carcass parameters at 41<sup>th</sup> day of the study. When overall results considered, there were no significant difference among treatments in terms of final live weight, mortality and European Production Efficiency Factor,EPEF (P> 0.05). While birds consumed diets that contain only phytase and phytase plus herbal Vit D<sub>3</sub> exhibited better FCR than control group without phytase(P< 0.05), no additional improvement was obtained with herbal Vit D<sub>3</sub> supplementation compared to only phytase. Higher level of herbal Vit D<sub>3</sub>, 0.2%, significantly decreased feed intake in low Ca and P diets compared to standart Ca, P diets.

Although lowering diet Ca and P level resulted significant reduction (P<0.05) in feed consumption compared to standart control(T1), FCR was significantly improved in birds received low Ca and P compared to T1. Besides inclusion of herbal Vit D3 or Phytase exhibited no improvements on carcass yield, drumsticks and breast meat ratio (P>0.05). Although tibia weight were not affected significantly, low level of Ca and P resulted significant decrease in ash and P content even supplemented with phytase and/or herbal Vit D<sub>3</sub> compared to standart recomended Ca and P level for broilers. Higher dose (0.02) of herbal Vit D<sub>3</sub> supplementation seems to have some adverse effect on final live weight, feed intake and tibia ash. These results indicates that herbal form of Vit D<sub>3</sub> at 0.01% could replace synthetic vitamin D<sub>3</sub> without any adverse effect on

performance, some carcass and tibia traits.

Key words: Panbonis, vitamin D3, broiler, performance

#### Introduction

Vitamin D can be derived from the diet or produced in the skin by means of sunlight. Dietary supplementation has become crucial due to intensive poultry farming does not allow to substantial synthesis of vitamin D. Vitamin D has been mostly provided to poultry by supplementation of the diet with synthetic forms of cholecalciferol (Vit D3). Cholecalciferol must undergo change to form 25 hydroxycholecalciferol (25-OH-D3) in liver and then in kidney 1,25 hydroxycholecalciferol (1,25-OH-D3) which is active form of vitamin D3. It is well known that 1,25 OH-D3 modulates Ca-P metabolism therefore suboptimal vitamin D3 levels in broiler diets adversely affect growth performance and bone development (1; 2). Herbal vitamin D3 sources has also been discussed as possible alternative to synthetic ones. The plant *Solanum glaucophyllum* was established that contains the active form of vitamin D3 in a glycosidic bound form in several research (3; 4). Studies have indicated that inclusion of *Solanum glaucophyllum* in diets improve growth performance and bone development of broilers and eggshell thickness of laying hens (5; 6;7). Phytase is another feed additives which an enzyme that improve performance and bone development of sy and became standart application in all poultry diets.

It has been considered that inclusion of *Solanum glaucophyllum* with phytase in broiler diets gives an opportunity to reduce dietary Ca-P levels (5). This study was conducted to test the effects of herbal source of vitamin  $D_3$  (Panbonis) with the presence of phytase at 2 levels of dietary Ca and P on performance, some carcass characteristics and tibia parameters of broiler chickens.

#### **Materials and Methods**

#### **Birds and Housing**

The research was carried out in Broiler Research House of Beypiliç Broiler Company in Bolu province, Turkey. 11200 day old mixed-sex Ross 308 chicks were weighed and randomly allocated to 56 floor pens ( $6.5 \times 2$  m). Rearing conditions were set according to breeder guidelines (Aviegen, Edinburgh, UK). Chicks had ad libitum access to feed and water.

#### **Experimental Design and Diets**

The research was conducted according to a completely randomised block design. Day old chicks were weighed and randomly distributed into 7 dietary treatments each has 8 replicates with 200 chicks in floor pens of experiment house through 41 days. Four phase feeding programme was administered through the experiment as starter (0-11 days), grower (12-25 days), developer (26-35 days) and finisher(36-41 days). Chickens were given starter (CP 23 %, Lys 1.43 %, ME 3025 kcal/kg), grower (CP 21 %, Lys 1.24 %, ME 3150 kcal/kg), developer (CP 19.60 %, Lys 1.09 %, ME 3225 kcal/kg) and finisher (CP 19.40, Lys 1.09, ME 3225 kcal/kg) diets that were formulated based on corn, soybean and wheat. Diets for each feeding period were formulated to be isonitrogenous and isocaloric and to meet or exceed breeder guidelines (Aviegen, Edinburgh, UK), but with different levels of avP, Ca and vitamin D3 (Table 1). 7 treatments was applied to evaluate the effects of panbonis on performance. First group was constituted as a control



containing regular Phosphorus (P), Calcium (Ca) and vitamin D3 level without phytase and herbal Vit D3. The second group was same to the control one except phytase supplementation. The third and fourth group were consisted of second group with lower vitamin D3 level and herbal Vit D3 supplementation at the rate of 100 and 200 g/ton respectively. Fifth group was preapared by decreasing P and Ca level of second group then was supplemented with 100 and 200 g/ton herbal Vit D3 reducing vitamin D3 level respectively to formulate sixth and seventh groups (Table 1).

**Herbal Vitamin D<sub>3</sub> (Panbonis®):** The herbal product contains 10 mg 1,25-Dihydroxycholecalciferol / kg in glycoside form and recomended to use 50 - 500 g per ton feed in addition to vitamin D from other sources. The product was supplied from Herbonis Animal Health, Basel, Switzerland.

**Phytase:** Phyzyme® XP (5000 FTU phytase/g) from Danisco Animal Nutrition, Marlborough, UK. 600 g phytase enzyme premix was included in 1 ton of diets to give 500 FTU/g.

Table	I. LAPCIN	mentar design									
Diets		Dietary factors		Starter		Grower		Developer		Finisher	
	Phytase FTU/g	Herbal Vit D <sub>3</sub> , ppm	Vit D <sub>3</sub> (IU)	Ca,%	avP,%	Ca,%	avP,%	Ca,%	avP,%	Ca,%	avP,%
T1	0	0	5000	1	0.50	0.90	0.45	0.85	0.425	0.85	0.425
Т2	500	0	5000	1	0.50	0.90	0.45	0.85	0.425	0.85	0.425
Т3	500	100	3000	1	0.50	0.90	0.45	0.85	0.425	0.85	0.425
T4	500	200	3000	1	0.50	0.90	0.45	0.85	0.425	0.85	0.425
T5	500	0	5000	0.82	0.41	0.78	0.385	0.76	0.36	0.72	0.36
T6	500	100	3000	0.82	0.41	0.78	0.385	0.76	0.36	0.72	0.36
Τ7	500	200	3000	0.82	0.41	0.78	0.385	0.76	0.36	0.72	0.36

 Table 1. Experimental design

#### **Data Collection**

All chicks were weighed at first, 11th and 41st days of the research. Feed consumption was recorded for 0-11 and 12-41 days. Mortality was recorded on a daily basis. FCR was calculated for 0-11 and 12-41 days. EPEF (European Production Efficiency) was also calculated at the end of the experiment. At 24th days of age 2 chicks from each replicates near to average weight of the pen were selected and killed for bone measurements. Both tibia of the each chicks was removed as a samples. Left tibias were analysed for tibia ash an phosphorus according to AOAC (2005). At the end of the trial 2 broilers from each pen was selected to assess carcass, drumsticks and breast meat yield.

#### **Statistical Analysis**

The data were analysed as a completely randomized block design with 7 dietary treatments and 8 replicates using the ANOVA procedure of the MINITAB 13. All percentage data were subjected to arcsine square root transformation. When significant differences among groups were found, means were separated using the Tukey HSD test.

# 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

#### Results

Phytase supplementation tended (P= 0.054) to reduce feed consumption however herbal Vit D3 enhanced feed consumption during the starter phase. Weight gain, FCR and mortality were not affected by dietary treatments in starter phase (P> 0.05). Although phytase supplementation to control diet improved FCR, no further improvements in FCR was detected by addition of herbal Vit D3 to diets with phytase during d 12-41 (Table 2). After starter, 12-41 d, chicks received T6 and T7 diets had significantly lower weight gain and feed intake (P< 0.05) than T2, T3, and T4(P<0.05).

When overall results considered, there were no significant difference among treatments in terms of final live weight, mortality and European Production Efficiency Factor, EPEF (P> 0.05). While birds consumed diets that contain only phytase and phytase plus herbal Vit  $D_3$  exhibited better FCR than control group without phytase(P< 0.05), no additional improvement was obtained with herbal Vit  $D_3$  supplementation compared to only phytase. Higher level of herbal Vit  $D_3$ , 0.2%, significantly decreased feed intake in low Ca and P diets compared to standart Ca, P diets (Table 3)

Although lowering diet Ca and P level resulted significant reduction (P<0.05) in feed consumption compared to standart control(T1), FCR was significantly improved in birds received low Ca and P compared to T1. Besides inclusion of herbal Vit D3 or Phytase exhibited no improvements on carcass yield, drumsticks and breast meat ratio (P>0.05), (Table 4). Although tibia weight were not affected significantly, low level of Ca and P resulted significant decrease in ash and P content even supplemented with phytase and/or herbal Vit D<sub>3</sub> compared to standart recomended Ca and P level for broilers(Table 5).

#### Discussion

Phytase addition to diets that contain regular Ca-P levels did not affect weight gain, FCR and mortality during the starter period (P > 0.05). Birds given the low Ca-P diets with phytase had similar weight gain and FCR when compared to birds consume regular Ca-P diets in the same period. This is in line with previous studies that indicate Ca-P level could be reduced by adding phytase without any depression in performance (8; 9). Phytase addition improved FCR but feed consumption and weight gain were depressed by reducing Ca-P level regardless of phytase or panbonis during d 12-41. Data showed that vitamin D3 levels might be reduced by herbal vit D3 addition without any adverse effect on weight gain and FCR during the starter phase however, from d 12-41 herbal D3 addition of reduced Ca-P diets inhibited weight gain and feed consumption. This study in line with Cheng et. al. (5) that reported no additional benefit in terms of FCR and weight gain by inclusion of Solanum glaucophyllum (dried leaves 7.5g/kg) with phytase (1200 FTU/kg). In the light of the results it could be assumed that phytase might cover effects of herbal vitamin D3 source. No difference observed between level of panbonis supplementation so it might be concluded there is no need 200 ppm panbonis addition to diets contained phytase. Inclusion of herbal D3 with phytase had no effect on carcass parameters. Tibia parameters of chicks fed diets that contain lower vitamin D3 level (3000 IU/kg feed) with panbonis were similar to that of the chicks fed diets contain regular vitamin D3 level (5000 IU/kg feed) without herbal source. This result indicated that herbal vitamin D3 could replace synthetic one in broiler diets without any adverse effect on tibia parameters. Although tibia ash (% of DM) and tibia P (% of dry-defatted tibia) were negatively affected by decreasing levels of Ca and P in the diets, performance of broilers were not affected in general throughout study. So



it could be claimed that Ca and P requirements of broilers needed to be carefully further studied, and re-evaluated.

#### Conclusion

Considering the performance, carcass and tibia parameters it might be concluded that herbal source of Vit D3 at 0.01% could be substituted for a portion of synthetic vitamin D3 without any adverse effect. However supplementation of higher dose at low Ca and P diets must be carefully considered and further studied because of negative impact on feed intake found in the present study. Phytase could allow formulating broiler diets both in standart and low dietary P or Ca without any adverse effect on performance and carcass parameters, but because of reduced tibia ash and P efficiacy of phytase and bone development should be taken in to account.

#### Acknowledgment

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Table 2. Effect of Panbonis, Phytase su	pplementation, Phosph	orus and Ca Level	l on Growth P.	erformance of B	roilers throug	h 0-11 and 12-4	41 days	
	11th Day Live	Weight Gain,	FCR	Feed Intake	Mortality	FCR	Feed Intake	Weight Gain
Treatments	Weight,g	0-11 Days,g	0-11 Days	0-11 Days,g	0-11	12-41 Days	12-41 Days,g	12-41 Days
1	309,52	266,01	1,237	328,68	4,56	1,729a	4014,51a	2321,57ab
2	313,11	269,52	1,209	325,61	4,03	1,705b	4031,31a	2364,22a
3	312,20	268,63	1,230	330,28	4,00	1,698b	3992,86a	2351,14a
4	312,82	269,28	1,236	332,35	4,25	1,691b	4018,98a	2377,09a
5	310,49	266,98	1,217	324,38	3,56	1,691b	3963,47a	2343,41ac
6	315,59	271,98	1,222	332,05	6,38	1,697b	3835,96b	2261,21b
7	306,68	263,13	1,251	328,98	4,88	1,691b	3863,32b	2284,37bc
P	0,11	0,12	0,59	0,49	0,23	0,004	0,005	0,005
Panbonis vs Phytase and Control								
Control(T1)	309,52	266,01	1,237	328,68ab	4,56	1,729a	4014,51	2321,57
Only Phytase(T2 and T5) Phytase Plus	312,01	268,47	1,210	324,67b	3,78	1,699b	4004,17	2355,90
Panbonis(T3,T4,T6,T7)	311,82	268,25	1,234	330,91a	4,87	1,694b	3927,78	2318,45
P	0.61	0.63	0.10	0.054	0.24	0.001	0.09	0.21
Phoshorus and Ca Level								
TI	309,52	266,01	1,24	328,68	4,56	1,729a	4014,51a	2321,57ab
T2,T3,T4	312,77	269,20	1,22	328,87	4,09	1,699b	4016,80a	2364,16a
T5,T6,T7	310,92	267,37	1,23	328,47	4,94	1,693b	3887,58b	2296,33b
P	0.36	0.38	0.63	0.99	0.37	0.001	0.001	0.003

# 3rd INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI



	Final Live	Weight	Feed		Mortality	
	Weight,	Gain	Consumption	FCR	0-41	
Treatments	day41,g	0-41 Days	0-41 days	0-41 Days	Days, %	EPEF
1	2731,1	2687,6	4506,8a	1,677a	10,81	340,3
2	2729,2	2685,6	4436,9ab	1,652b	10,61	345,9
3	2729,1	2685,6	4428,1ab	1,649b	10,31	347,9
4	2742,1	2698,6	4432,8ab	1,643b	11,44	346,6
5	2711,7	2668,2	4378,1bc	1,641b	9,56	350,1
6	2727,7	2684,1	4413,6bc	1,644b	12,56	339,9
7	2681,0	2637,5	4336,0c	1,644b	12,06	335,9
Р	0,28	0,28	0,01	0,003	0,31	0,24
Panbonis vs Phytase and Control						
Control(T1) Only Phytase(T2 and	2731,11	2687,60	4506,82a	1,677a	10,81	340,29
T5) Phytase Plus	2719,02	2675.48	4404,14b	1,646b	10.15	347,63
Panbonis(T3,T4,T6,T7)	2720,00	2676.43	4402,62b	1,645b	11.59	342,56
Р	0.85	0.85	0.01	0.001	0.17	0.25
Phoshorus and Ca Level						
T1	2731,11	2687,60	4506,82a	1,677a	10,81	340,29
T2,T3,T4	2732,89	2689,32	4433,22ab	1,648b	10,76	346,65
T5,T6,T7	2706,81	2663,26	4375,88b	1,643b	11,39	341,96
Р	0.23	0.23	0.002	0.001	0.67	0.28

**Table 3**. Effects of Panbonis, Phytase Supplementation, Phosphorus and Ca Level on Growth

 Performance of Broilers through 0-41 Days

**Table 4**. Effect of Panbonis and Phytase Supplementation on Some Carcass Characteristics % of Live Body Weight

Treatments	Carcass Yield	Drumsticks	Breast meat
1	71,78	31,59	32,58
2	71,89	32,03	32,08
3	72,13	32,00	32,31
4	71,87	31,36	32,81
5	72,67	32,18	32,46
6	72,28	31,57	32,86
7	71,63	31,65	32,21
Р	0,73	0,32	0,72
Panbonis vs Phytase and Control			
Control(T1)	71,78	31,59	32,58
Only Phytase(T2 and T5)	72,28	32,11	32,27
Phytase Plus			
Panbonis(T3,T4,T6,T7)	71,98	31,64	32,55
Р	0,62	0,12	0,67

	Dry-	• supprenienter		Tibia P, %	
	defatted			of	Tibia ratio,%
	tibia	Tibia ash,	Tibia P, %	dry-defatted	in 24 day's
Treatments	weight, g	% of DM	of ash	tibia	live weight
1	3,97±0,16	41,83±0,44a	17,13±0,17	7,18±0,12a	0,31±0,011
2	4,00±0,11	40,59±0,30b	16,94±0,14	6,89±0,09b	$0,30\pm0,005$
3	$3,74\pm0,11$	40,60±0,28b	16,97±0,09	6,90±0,06b	$0,29\pm0,004$
4	3,93±0,13	40,60±0,28b	$17,03\pm0,07$	6,92±0,07b	0,31±0,010
5	3,83±0,11	39,95±0,38bc	17,01±0,09	6,81±0,05b	$0,30\pm0,005$
6	$3,60\pm0,11$	39,77±0,25bc	$17,05\pm0,08$	6,80±0,06b	$0,29\pm0,004$
7	3,73±0,11	39,64±0,31c	$16,98\pm0,06$	6,74±0,05b	$0,30\pm0,005$
Р	0,21	0,002	0,92	0,01	0,44
Panbonis vs Phytase and	Control				
Control(T1)	3,97±0,16	41,83±0,44a	17,13±0,17	7,18±0,12a	0,31±0,011
Only Phytase(T2 and					
T5)	3,91±0,08	40,27±0,20b	16,97±0,08	6,85±0,05b	$0,230\pm0,003$
Phytase Plus					
Panbonis(T3,T4,T6,T7)	$3,75\pm0,06$	40,15±0,17b	$17,00\pm0,04$	6,84±0,03b	$0,30\pm0,01$
Р	0,14	0,000	0,52	0,001	0,31
Phoshorus and Ca Level					
T1	3,97±0,15	41,83±0,44a	17,13±0,17	7,18±0,11a	0,308±0,011
T2,T3,T4	$3,88\pm0,07$	40,59±0,18b	16,98±0,05	6,90±0,04b	$0,297\pm0,004$
T5,T6,T7	3,72±0,06	39,79±0,16c	17,01±0,04	6,78±0,04c	0,296±0,003
Р	0,12	0,000	0,50	0,000	0,31

#### Table 5. Effects of Panbonis, Phytase supplementation and Ca-P level on Tibia Parameters



# O<sup>26</sup> Strategies to Save Methionine in Broiler Diets

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#### Abstract

Feed cost is the largest and most important input of broiler production. The feed cost in broiler production constitutes approximately 65-85% of the total production cost. High feedstuff prices, dependency on import, problems in obtaining the feedstuff, increasing mixed feed prices negatively affect the broiler production cost. In poultry production, Methionine is a sulfurous, essential and limiting amino acid. Methionine, cystine and cysteine belong to sulfurous amino acids. Since methionine prices increased 3 times in recent years, this resulted in development of new strategies that saves and uses less synthetic methionine by nutritionists. In this review, information on possibilities of using different methyl sources replacing methionine in broiler diets are discussed.

# O<sup>27</sup> Effect of Relative Humidity During Incubation (0-18.5 D) on Hatchability and Broiler Performance

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#### Abstract

This experiment was conducted to determine the effect of relative humidity during incubation (0-18.5 d) on hatchability and broiler performance in two Experiments. The broiler breeder ages were 31 wk and 60 wk in Experiment 1 and 2, respectively. Egg weight loss in high and normal RH groups, were 8.5 %, and 12.4 % in Experiment 1 and 9.6 %, and 11.3% in Experiment 2, respectively. Although egg weight loss decreased significantly with higher RH treatment (P<0.05), Relative humidity treatment had no effect on fertile hatchability and broiler performance in both Experiment (P>0.05).



# O<sup>28</sup> New Aspects and Changes in Broiler Breeder Nutrition To Improve Performance and Hatchability

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#### Abstract

Nutrition of the modern broiler breeder is challenging. It has a very high genetic growth potential which may negatively correlate with reproductive traits. Controlled growth, uniformity and optimal carcass composition with high enough fat reserves during rear are the key factors which influence future reproductive performance of the flocks. Careful feeding to peak, and close follow-up of the condition of the birds and feed adjustments accordingly during the laying period, both help in achieving good performance and incubation results.

#### Background

Broilers are continuously growing faster, they are more feed efficient and have a higher genetic potential for breast meat production. As an example, the 35d live weight of Ross 308's has increased from 1900 to 2400g and its breast meat yield from 18.5 to 21% from 2006 to nowadays. Because of high breast meat growth, modern broilers are leaner than in the past and their fat content is lower. For comparison, from the year 1980 up to 2000, the fat pad of broilers from the same breed as a percentage of body weight decreased by 50%, from 5.68 to 2.67% (Eitan et al., 2014). With a propensity to deposit muscle rather than fat, there may not be enough energy stored in the body to mobilize in times of energetic shortage, and as a result broiler breeder hens may have difficulties with early chick quality and long-term maintenance of lay (Renema et al., 2014).

Although the growth rate and reproductive characteristics are negatively correlated (Leeson and Summers, 2005), the modern breeder hen still has the potential to yield at least 145 chicks over its 40-week productive life. It is, however, imperative that proper management is maintained during rear because the performance of the flock during this period will influence reproductive performance during lay. Delivering the correct level of nutrients to obtain maximal productivity, while at the same time moderating the bird's inherent desire for growth, presents a significant challenge. Therefore, feeding the modern broiler breeder requires a holistic strategy of nutritional parameters and feeding programs to be correctly established (Silva, 2014).

#### **Proper Rearing is Crucial**

The main factors influencing the reproductive performance of the flock are established during the rearing period, and one of the most important one is determining the correct body composition and the hen's fat reserves to fuel subsequent egg production. Nutrition from hatch to 4-5 weeks of age must focus on the proper development of skeletal, intestinal, cardiovascular, and immune systems along with a highly uniform development within the flock (Silva, 2014). The feed must provide essential amino acids, minerals, trace elements and vitamins in sufficient quantities. Recommended protein content of the starter diet is 19% with a digestible lysine 0.95%, and containing 2800 kcal of AME/kg (Ross, 2013). In the beginning, the birds are fed *ad libitum*,

but typically from 3 - 4 weeks of age feed will be given restrictively. Daily amounts needed are affected by the dietary energy concentration and the internal house temperature.

Breeding companies have set optimal growth and weight targets for each breeder strain and strain cross. In practice, feed restriction has been used to limit excessive weight gain and maximize egg production and fertility. Female broiler breeders that are full fed have decreased total egg output (Robinson et al., 1991). In a trial by Hocking et al., (2002), the performance of conventionally restricted birds, measured as the numbers of chicks produced per unit of food consumed, was 4-fold higher than that of *ad libitum* fed birds. Indeed, benefits of feeding the breeders in a restricted manner include reduced body weight, delayed sexual maturity, improved ovarian function with fewer multiple ovulations, and higher egg production with better persistency and longer sequences, reduced number of abnormal eggs, and better liveability during laying period (Renema and Robinson, 2004). Due to the increased growth rate of broilers, the recommended growth profile of broiler breeders has been increased as well, but to a lower extent than the increase in growth rate and growth potential. In practical terms, this means more severe control of feed intake for modern broiler breeders than in the past (Renema et al., 2007). To illustrate the need for feed restriction, especially during the rearing period, commercial female broilers fed ad *libitum* achieve a 3 kg weight at 47d of age, while the Ross 308 breeder composed of the same genetic background should achieve this weight only at 25 weeks of age.

Rearing feed is formulated with an energy level in the range of 2,600 to 2,800 kcal ME/kg (Ross, 2013). Feed nutrients have to be adjusted according to energy in order to maintain an optimal energy: protein ratio. Protein contents during the rearing diet oscillate between 14 and 15%, while recommended digestible lysine contents do so at 0.52% or 0.61%, for low and high energy diets, respectively. Excess protein and amino acid intake will lead to over-fleshed birds with low level fat reserves. In a trial of van Emous et al. (2013) feeding a low protein diet during rearing decreased breast muscle and increased abdominal fat pad at the onset of lay, while improving uniformity. When a high energy diet is fed, flock uniformity may be more difficult to achieve because a smaller amount of feed is needed to meet the energy requirement of the bird, and feed clean-up times are short (Silva, 2014).

Good quality low density rearing diets have many advantages, including reducing the hen's stress as a result of a prolonged feeding time (Jong et al., 2005). A higher energy to protein ratio prolongs the time spent on eating and reduces stereotypic object pecking in breeder pullets (van Emous et al., 2013). If included at the correct particle size, fibre rich raw materials will provide sufficient structure to stimulate gizzard development which will regulate the flow of nutrients into the small intestine, optimising digestive processes and nutrient utilisation (Hetland et al., 2005). Moreover, low-density diets may promote satiety through a more filled gastrointestinal tract, and thus feelings of hunger may be reduced (Jong et al., 2005).

Ross 308 recommended feeding program results in a cumulative consumption of energy and protein at 20 weeks of age of about 22,260 kcal of ME and 1230g of protein. When offering a low density grower ration, feed allocation must be increased to cover the daily requirements of the bird. Increments in the amount of feed must be aligned with the standard daily energy requirements profile, to allow birds to achieve the correct body weight gain at target age. To help with this, breeding companies have proposed daily energy allowances for the rearing period (Figure 1).





Figure 1. Ross 308 female Daily Energy Intake Profile and Body Weight during rearing period.

#### Flock Uniformity is A Must

The uniformity of a pullet flock is essential. If the uniformity is low (high CV %), the birds will come into lay unevenly and cannot achieve optimum reproductive performance. Maintaining good uniformity is even more important with modern broiler breeders because they are able to rapidly consume their allocated feed and deposit protein with limited body fat deposition. For uniformity, a good management practise is to implement grading for body weight. Grading broiler breeder pullets according to body weight as a part of a restricted feeding program can result in a more uniform flock at 20 weeks of age (Petite et al., 1981).

After grading, a specific amount of feed is provided to each population so that the weight difference between each population is reduced weekly, and flock uniformity is maximized at the onset of lay. Adequate feeding must occur during the rearing period in order to synchronize sexual maturity, achieve consistent and uniform growth and maintain future egg production and chick quality (Silva, 2014). First grading of males and females should be done at 4 weeks of age. After grading, bodyweight profiles are revised to achieve target body weights by 9 weeks of age. Daily feed allocations should be adjusted to achieve the revised bodyweights and maintain uniformity. The weight gains need to be monitored weekly.

#### **Pre-laying Phase**

The pre-laying phase is a crucial stage in the preparation of the broiler breeder female for sexual maturity and egg production. At this time, the bird must receive the right feed increments in order to successfully complete the growth phase. The classical concept of pre-layer diet containing 1.2-1.5% calcium is to improve the birds' preparation for the laying phase by increasing calcium deposition in medullary bone and the rate of bodyweight gain. Currently, the use of pre-layer diets can have a different connotation according to the dietary energy concentration used in the developing period. In case the grower diet has low content of AME (2600 - 2650 kcal/kg), a pre-layer diet with higher ME content will allow a smoother energy transition into the production phase, promoting, even with small weekly increases in feed amounts, proper weight gain, breast

conformation and fat reserve deposition (Silva, 2014). In addition, feeding just a low-energy grower diet until lay might pose a problem because breeders should not be subjected to a step down in feed allocation prior to peak production (Leeson and Summers, 2005). In the event that the energy content of the diets used in rear and in lay are similar, the use of a pre-layer diet might be unnecessary (Silva, 2014).

#### **Production Phase**

The objective during this phase is to achieve maximum number of fertile hatching eggs with emphasis on chick quality during the first weeks of production, and then hatchability during late production. Figure 2 shows the importance of each 5-week period during production on total egg output. The first phase of production (25 - 40 weeks) is responsible for almost 45% of total egg output and the late phase of production (>50 weeks) for more than 25% of total egg production.



Figure 2. Important aspects during egg production (%) of broiler breeders.

The priority in nutrition during this period is to ensure the female receives enough nutrients for body weight maintenance, growth, egg production and activity, simultaneously. Between weeks 20 - 30 the flock still increases its body weight by 40%. The nutritional requirements of the female must be met by providing an adequate daily energy allowance, while maintaining the correct relationships between energy, protein and essential amino acids, and energy with vitamins and minerals (Silva, 2014).

Small deviations in feed allocation may adversely affect the production of eggs and chicks (Robinson et al., 1993). Assuming that birds are continually producing yolk precursors within a follicular hierarchy, the feed allocation from onset of lay to peak production is a key point for maintaining weight gain, achieving standard egg size and avoiding metabolic disorders.

As described in the Aviagen Parent Stock Management Handbook (2013), peak feed should be given when birds achieve ~60% of hen day egg production. A small and constant increase in feed amount should be given from 5% of hen day egg production onwards. When flocks have low fat reserves (< 2.5% of abdominal fat pad as % of live weight) (Sun et al., 2006) peak feed should not be delayed beyond 70% of hen day egg production. If peak feed is delayed, the existent fat reserves may be utilized during peak of egg production. In an attempt to replenish that, feed reduction post-peak might be compromised, which might impact the control of body weight and



egg weight, possibly impacting persistency of egg production (Silva, 2014).

As described before, modern broiler breeders, as a result of their genetic background, are likely to have reduced energy stores available as fat reserves if their feed intake is below their needs. Energy deficiency will affect the immunological system, feathering status, and egg production and persistency. Besides energy, a proper nutritional balance that avoids or prevents the onset of metabolic problems, maximizes egg shell quality, controls egg size properly, and guarantees the right transfer of nutrients to progeny also plays an important role (Silva, 2014).

After peak, the birds need less energy and nutrients for egg production. Oversupply of nutrients will result in increased growth and obesity, quickly leading to reduced egg production (Leeson and Summers, 2005). Thus, daily nutrient allocation has to be reduced after peak. This may be done by decreasing the feed allocation or by reducing the nutrient content of the diet. Reducing feed post-peak is not a simple task, and the time and amount of feed to be reduced depends on several factors including body weight curve and body weight gains from start to production, daily egg production and trends, energy stores as fat reserves, egg weight trend, health status of the flock, ambient temperature, feathering status and flock history (Silva, 2014). During post-peak is important that the birds gain some weight as this helps in maintaining the egg laying persistency, fertility and hatchability.

Proper control of daily protein and amino acid intake in broiler breeders is not only needed during rear when it affects body composition (van Emous et al., 2013), but also in lay when it may have an impact on weight gain and egg size (Joseph et al., 2000). The protein and amino acid levels in the diet need to be related to dietary energy content. Considering the energy requirement of 460 - 470 kcal/bird/day at peak (at an environmental temperature of  $21^{\circ}$ C) and an expected consumption of 24 - 25g of protein/d, a Breeder 1 diet formulated with 2800 kcal of AME/kg and 15% crude protein will likely result in excellent egg production peak and good egg size at the beginning of production (Aviagen 2014, unpublished). The use of diets with higher protein for extended periods may result in body-weight control problems, and often results in overweight birds and increased egg size (>65g) just after 40 weeks of age. The hatchability of heavy eggs is reduced (Shafey, 2002).

Ross Parent Stock Nutrition Specifications (2013) recommends two-phase feeding program for the laying period; a Breeder 1 diet with higher nutrient content from 5% of production to 35 weeks of age, and Breeder 2 thereafter, with lower protein and amino acid content up to depletion. The energy recommendation for the diets remains the same.

#### Vitamins and Minerals

Proper dietary vitamin and mineral supplementation in the laying phase is important for promoting good fertility, hatchability and chick quality. Adequate levels of vitamins and minerals are needed for supporting normal embryo development. Deficiencies may cause embryo mortality, malformation or other abnormalities.

#### **Feed Physical Form**

Physical quality, fibre and energy content of the feed have been shown to influence eating-up time, gizzard function, feather pecking and plumage condition in laying hens (van Krimpen et al., 2005). In an unpublished trial of Aviagen (2014), breeders fed a coarse mash diet formulated

according to recommendations had better performance than breeders fed a diet in crumble form. Whole cereal inclusion seemed to improve hatchability and chick output. Gizzard size of breeders fed diets containing whole cereals was bigger than gizzard of breeders fed crumble diets.

#### Hatchability

From a nutritional point of view, hatchability can be influenced by both male and female breeders, the nutrients deposited in the egg for the embryo, and certain physiological egg characteristics that can affect gas and water exchange during incubation (Leeson and Summers, 2005). Van Emous et al. (2013) compared the effect of high and standard growth profiles (2400 vs 2200g live weight at 20 weeks of age) and 3 dietary protein levels (14.1, 13.3 and 12.5%) during rearing on fertility, hatchability, embryonic mortality and chick quality. A higher growth pattern during rearing period increased fertility, decreased embryonic mortality and improved offspring performance in young breeders (29wks). Results of the incubation trials of 33 and 37 weeks did not show any difference in fertility or embryonic mortality. Decreased protein level had a less pronounced effect on these traits. In another trial (van Emous et al., 2015), a low-protein diet (11.3% vs. 14.1%) fed to breeders during rearing changed the body composition with positive effects on incubation traits during the first phase, and an improved egg production during the second phase of lay. Reducing or increasing dietary energy level compared to the standard (2,800 kcal/kg) during the first phase of lay resulted in slightly negative effects on production while a high-energy diet (3,000 kcal/kg) during the second phase of lay showed positive effects on hatchability, embryonic mortality and number of settable eggs.

Maintaining good egg shell quality is important during the entire laying period. Poor shell quality could mean a potential loss of settable eggs and reduced hatch of fertile eggs. Overfeeding energy and protein may reduce fertility both in females and males (Leeson and Summers, 2005). Pearson and Herron (1982) reported breeders consuming 27 g of protein/day produced an increased number of dead and deformed embryos, and the hatchability of fertile eggs was decreased compared with breeders consuming 23.1 g of protein/day. Lopez and Leeson (1995) showed a decrease in fertility in hens fed higher CP levels (16% vs. 10 to 14%).

Replacing part of the trace minerals with organic forms (Favero et al., 2013; Urso et al., 2015), or vitamin D3 with HyD (Saunders-Blades and Korver, 2015), increasing the dietary vitamin E content (Urso et al., 2015), have all been reported to improve hatchability, at least in young breeders. Some feed additives like L-carnitine (Leibetseder, 1995; Aviagen 2014 unpublished) and *Saccharomyces cerevisiae* yeast product (Kidd et al., 2013) may positively affect hatchability.

#### Male Feeding

The use of a single feed for both sexes is a widespread practice in the laying period. However, the use of a specific male diet in the laying period has been shown to be beneficial to the maintenance of male physiological condition and fertility by direct nutritional effects, either by avoiding excess levels of protein and/or calcium (Moyle et al., 2011). If a separate male diet is used, it should be introduced at 25-26 weeks. A separate male diet with lower protein and amino acid levels can prevent excessive breast muscle development and ensure proper feed allocation during the production period improves fertility (Romero-Sanchez et al., 2008). Feed additives like organic selenium, L-carnitine, ascorbic acid, and creatine may be beneficial for sperm quality. Male diets



are typically formulated with a range of 2600 to 2850 kcal AME/kg.

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# O<sup>29</sup> Enterococcus Spp. from Turkey Retail Meat Samples Resistant to Vancomycin and Their Antibiotic Resistance Profiles\*

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#### Abstract

The objectives of this study were to isolate and identify *Enterococcus* spp. from turkey retail meat samples by classical culture method and using biochemical tests, to verify the isolates by PCR (Polymerase Chain Reaction), to evaluate the antibiotic resistance profiles of the verified *Enterococcus* spp. isolates with 8 different antibiotics including eritromisin, kloramfenikol, gentamisin, penisilin, streptomycin, vancomycin, linezolid, quinupristin-dalfopristin according to CLSI (Clinical and Laboratory Standards Institute) standards, to determine the molecular characterization of the isolates for *van* A and *van* B resistance phenotypes with multiplex PCR, to determine MIC (Minimum Inhibitory Concentration) values of all the isolates determined as vancomycin resistant by E test. For this purpose, 100 turkey retail meat samples collected from Ankara markets were analyzed. 20 % of the isolates were determined as resistance to 5 or more antibiotics and 88 % (44 isolates) were resistant to vancomycin. From total of 50 isolates 25 of them were found positive for *van* A and *van* B genes. MIC results of the vancomycin resistant isolates were varied between 0,25 ile 16 µg/ml.

\*This study was summarised from PhD thesis of first author

# O<sup>30</sup> Recent Developments in the Use of Exogenous Enzymes for Broilers\*

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#### Abstract

The global feed enzyme market today is worth almost \$1bn and has grown remarkably quickly since its infancy in the 1980s. The vast majority of poultry, and to a lesser extent swine, diets now contain at least one feed enzyme (typically phytase) and many contain two or more. The principal motivator for enzyme use is feed cost reduction but increasingly performance advantages are sought, especially where there are constraints on space in formulation. Current trends in enzyme research include increasing dose rates for phytase, exploration of optimal enzyme combinations, integration of raw material quality with enzyme use, emergent mono-component protease technology and renewed interest in enzymes (especially xylanases) as microbial modulators. The present paper will discuss some aspects of these and how enzyme technology may develop in the future.

#### **Reducing Returns and Ingredient Quality**

One of the most significant challenges facing expansion of existing feed enzyme markets is competition from within the feed enzyme sector itself. Feed enzymes are registered and marketed principally as digestibility enhancers with focal nutrients including energy, calcium (Ca), phosphorus (P) and amino acids. The degree to which these nutrients are digested by the animal influences the magnitude and the consistency of the response to the feed enzyme (Cowieson & Bedford, 2009; Cowieson, 2010, Fru-Nji et al. 2011; Cowieson & Roos, 2014). As apparent ileal digestibility of the focal nutrient approaches 95-100% the opportunity for the enzyme to improve digestibility further is disproportionately reduced. This represents a challenge for continued expansion of the feed enzyme market to 2020 as diets that already contain phytase, xylanase/glucanase and protease may return digestibility of amino acids, P, Ca and energy that approach physiological limits. These constraints are exacerbated by improved husbandry practices, improved genetics of the animals and the ingredients themselves and of course increased knowledge of nutrition. Thus, to maintain the current growth in the feed enzyme market up to and beyond 2020 enzyme technology must move into new areas, to enhance performance, reduce mortality, reduce environmental impact of intensive animal production and to increase enteric resilience. Additionally, monitoring of raw material quality (at the level of the digestibility of the focal nutrients) is logical to allow enzyme solutions to be tailored to the nutritional gaps presented by the ingredients in the diet.

#### **Nutritional Obscurity**

Optimal application of feed enzymes is reliant on a well-defined starting point and clarity and agreement in nutritional definitions and targets. However, several areas of nutritional obscurity persist in diet formulation including key nutrients such as calcium and energy. For example, formulation of diets on a total calcium basis results in considerable confusion in interpretation of the effects of phytase and the appropriateness of ratios to available phosphorus. Additionally, formulation of diets using a metabolisable energy system that does not consider the source of the energy contributes to difficulty in appropriately assigning enzyme effects that may originate from various macro-nutrients. Recent research in a range of species from insects and fish



to humans has shown clearly that energy per se is usually not a priority target and animals will over- or under-consume total energy depending on the proportional composition of the energy in the diet they are offered (Simpson & Raubenheimer, 2012). These concepts have profound implications for the appropriate use of feed enzymes as increasing energy digestibility as a target is too crude and even if successfully delivered will have an unpredictable effect on animal performance. Rather than assigning energy matrix values to feed enzymes it would be more useful to assign matrix values for carbohydrate, amino acids/protein, lipid and where necessary for various macro- and micro-minerals. In this way feed enzymes may be formulated into diets to displace the most appropriate energy-yielding macronutrient and not, as is quite often the case, only fat. Simpson & Raubenheimer (2012) elegantly review these concepts as they relate to animal and human biology in general and show clearly that in most species it is digestible protein and not energy that is more closely regulated. This 'protein leverage' effect has substantial implications for monogastric production animal nutrition in general and also how we use feed enzymes to either reduce diet cost or to improve FCR. If broilers eat to defend a protein intake target (Shariatmadari & Forbes, 1993; Simpson & Raubenheimer, 2012) then we must consider macronutrient proportionality and the effect a particular enzyme has on the digestibility of amino acids, carbohydrate and lipid individually and not only on the (in) consequential effect that this may have on energy digestibility. In the opinion of the author this will have a profound impact on how enzymes will be used and optimized and on how enzyme admixtures may be assembled.

#### Phytase Super-Dosing and *Myo*-inositol

Phytase was first commercialized in the early 1990s and the recommended inclusion concentrations were associated with P release values of around 1g/kg, representing approximately 30-40% phytate hydrolysis (Selle & Ravindran, 2007). More recently so-called 'second' or 'third' generation phytases have been developed that are capable of hydrolyzing 60-70% of dietary phytate at similar unit for unit dosing (Cowieson et al., 2011). These improvements in phytase technology are associated with various characteristics of the phytase protein including improved gastric resilience, more optimal pH profiles and better thermal tolerance. Additionally, phytases are applied more effectively today than in the early 1990s as information emerged regarding the antinutritive effect of phytate and the role of Ca, sodium (Na) and the interactions between phytate and proteins (Cowieson et al. 2010; Selle et al., 2012), allowing more appropriate diet formulation. Currently there is considerable focus on 'super-dosing' phytase, a term that is not universally understood nor clearly defined. In the opinion of the authors 'super-dosing' may be considered to be addition of 2x or 3x conventional doses of phytase where a nutrient matrix is applied only to the standard dose, with the additional phytase being added 'over-the-top' as a growth promotant. This topic has been recently reviewed (Cowieson et al., 2011) and the growth promoting effect of unconventionally high doses of phytase are thought to be associated with a more complete and rapid removal of reactive phytate from the digestive mileu and a more persistant solubility of lower esters of inositol phosphate in the small intestine. The latter is a prerequisite for mucosal or systemic phosphatase-mediated complete dephosphorlyation of the residual inositol polyphosphates and generation of *myo*-inositol. Importantly, substantial increases in plasma *myo*-inositol concentrations in pigs supplemented with microbial phytase has recently been established (Guggenbuhl et al., 2013).

*Myo*-inositol is one of nine possible stereoisomers of cyclohexane and is the core of phytic acid. It is loosely classified in nutrition as a member of the B vitamin family and is not considered

# 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

to be essential for growth. However, recent work (Zyla et al., 2004; Cowieson et al., 2013; Zyla et al., 2013) have shown growth-promoting effects of *myo*-inositol when added to broiler diets, either with, or without, phytase but this has not been replicated in layers (Zyla et al., 2012). Importantly, recent work in mice has established that orally-administered *myo*-inositol stimulated the translocation of glucose transporter 4 (GLUT4) in the plasma membrane, an effect that is insulin mimicking (Yamashita et al., 2013). Thus, myo-inositol may be considered to be insulin mimetic, reducing blood glucose concentrations by stimulation of GLUT4 translocation (at least in mammals). This stimulation of GLUT4 translocation is usually achieved by insulin which binds to the receptor, translocating GLUT 4 from intracellular storage to the cell surface via a cascade involving phosphatidylinositol-3 kinase (Dang et al., 2010). However, several studies have now demonstrated the insulin-mimetic effects of inositol isomers, including myoinositol and the resulting stimulation of GLUT4 (Dang, et al., 2010; Yamashita et al., 2013). In addition to the obvious implications for human nutrition and in particular diabetes management, these effects may be responsible for the growth promoting effects of super-dosing phytase in broilers and pigs, mediated via increased glucose uptake into various tissues and inhibition of gluconeogenesis and muscle catabolism. The effects of phytase on inositol and glucose transport may explain some of the observed interactions between phytase and sodium and sodium-dependent transport systems observed in the recent literature (summarized in Cowieson et al., 2011). These mechanisms may differ between pigs and poultry as poultry (and other birds) do not express GLUT4 (Carver et al., 2001; Tokushima et al., 2005; Sweazea & Braun, 2006). However, as birds respond to insulin (Tokushima et al., 2005) and also inositol (Cowieson et al., 2013) it is likely that they do possess an insulin responsive glucose transport mechanism and the net effect may be similar across species.

Thus, super-dosing phytase may have three central mechanisms of effect including increased available P concentrations (which may be of value, especially in neonates), reduced antinutritive effects of phytate by more complete and rapid dephosphorylation, and finally increases in plasma *myo*-inositol concentrations which facilitate glucose uptake and muscle development.

#### Enzymes, Gut Health and the Microbial Flora

Since exogenous enzymes were first commercially introduced in the mid-1980s there have really only been 3 distinct enzyme classes that have gained any traction. These enzymes include structural fibre degrading enzymes (dominated by xylanase), phytase and more recently, protease. Other activities such as lipase, amylase and other carbohydrases such as pectinase, galactosidase and mannanase are less heavily researched or understood. The obvious question for the future of feed enzymes is which genuinely new activities may be developed, successfully, over the next decade or so? This is, of course, not only a hard question to answer but impossible to do so entirely openly without breaching confidentiality and intellectual property agreements. However, starting by considering which new enzyme may be of interest is inappropriate. It is more useful to consider where the current limitations are in nutrition and so where an intervention may have most value. One area that has a great deal of potential is microbial management and this is an area where there is only little momentum now (for feed enzymes) compared with a decade ago (Bedford & Cowieson, 2012). Using enzymes specifically to modify the microflora or to mitigate enteric infections has been demonstrated to be possible but remains an elusive area in praxis. However, recent work has suggested a more intricate link between the microbial flora in the distal GI tract and enzyme responses than has been previously considered (Cowieson & Masey-O'Neill, 2013a). These responses hinge on the generation of fermentable oligomers in



the GI tract which are fermented in the caecum, triggering the ileal brake mechanism. This effect has been recently demonstrated by measuring one of the hormones responsible for regulating the ileal brake mechanism, peptide YY (PYY; Singh et al., 2012). It is evident that xylanase addition can increase PYY concentration in the serum of broilers, possibly indicating a modifying effect of xylanase on gastric residency. Further evidence for this mechanism is presented by Cowieson & Bedford (2009) where xylanase effect on ileal amino acid digestibility was found to be a constant fraction of the undigested amino acids in the diet. This generic effect suggests that de-caging is not a major mechanism for xylanase or those amino acids most often found in the aleurone layer of e.g. wheat (such as Arg) would be disproportionately advantaged. That all amino acids are advantaged to the same degree (relative to the undigested fraction) suggests a mass effect, such as changed gastric residency. Enzymes that can directly hydrolyse undesirable microorganisms is also possible but again is under-researched in a feed context.

#### **Enzymes and Gut Health**

Gut-health is a widely used term and though it is not clearly defined, it is understood to mean a gut which is generally free from any adverse exogenous challenge and is functioning optimally. There are many diet, microbial and environmental factors that will reduce the health of the GI tract of pigs and poultry (Klasing, 1998). Such factors include certain types of fibre, trypsin inhibitor, phytate, lectins, undigested protein in the distal GI tract, pathogenic and putrefactive microorganisms, diets with poor nutrient balance, temperature stresses, poor water quality, certain vaccination programs and many others. Enzymes have been shown to beneficially influence several of these metrics of gut health and so improve the enteric resilience of the animal (Bedford & Cowieson, 2012) but the mechanisms involved are not fully explained. Enzymes may increase both the rate and completeness of protein, starch and fat digestibility, shifting the site of metabolism more proximally in the GI tract. This will reduce substrate availability for the microflora in the ileum and caecum and also reduce the length of the intestine with a consequential sparing effect on maintenance energy demand. Enzymes may also reduce the negative effect of various dietary antinutrients such as phytate (Cowieson et al., 2004) and fibre (Angkanaporn et al., 1994). Additionally, enzymes improve the digestibility of a range of conditionally essential nutrients such as Gly, Ser, Pro, Phe, Zn and Fe which may become particularly important during disease challenge or post-vaccination recovery (Klasing, 1998). Thus, enzymes may impart a general 'sparing' effect on nutrient requirement, reducing visceral mass, improving immune competence and enteric resilience in addition to the more conventional influences on nutrient digestibility.

#### Conclusions

It can be concluded that development of new enzyme technology over the next decade will be more challenging than has been the case in the last decade. Not only is the ileal digestibility of the nutrients that drive diet economics relatively high but there is increasing competition from other micro ingredients and a more challenging regulatory environment. It is likely that ingredient quality assessment will become more widespread in order to strategically align enzyme choice with the undigested nutrients in the feed. Furthermore, new enzyme candidates will emerge in novel areas such as microbial and environmental management that do not compete directly with incumbents for space in feed formulation. Finally there will continue to be progress in existing enzyme sectors including new xylanases, glucanases, proteases and phytases that have enhanced characteristics compared with existing products. Feed enzymes have a bright future and allow nutritionists more freedom in formulation and contribute tangibly to sustainability of animal protein production. The next decade will be an exciting one for this relatively new technology as enzyme suppliers work closely with customers to deliver innovative solutions to improve the profitability of their enterprises.

# \*: This paper is based on an abstract previously presented at the 5<sup>th</sup> International Broiler Nutritionists Conference, Queenstown, New Zealand, 2014.

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# O<sup>31</sup> Nitrogen Corrected Metabolizable Energy Value of Camelina Meal for 4-wk old Broiler Chickens

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#### Abstract

Limited information on nutritional characteristics on camelina meal (CM) for broiler chickens limits its use in diets of broiler chickens. The objectives of this study were to determine the ileal digestible energy (IDE), ME, and ME<sub>n</sub> contents of two different camelina meal (CM1 and CM2) samples for 4-wk-old broiler chickens using the regression method and to determine glucosinolate compounds in the CM samples. The CM1 and CM2 were incorporated into a corn-soybean meal-based reference diet at 3 levels (0, 100, or 200 g/kg) by replacing the energyyielding ingredients. These 5 diets (reference diet, and 100 and 200 g/kg camelina meal from each of CM1 and CM2) were fed to 320 male Ross 708 broilers from d 21 to 28 post hatching with 8 birds per cage and 8 replicates per treatment in a randomized complete block design. Excreta were collected twice daily from d 25 to 28, and jejunal digesta and ileal digesta were collected on d 28. The total glucosinolate content for CM1 and CM2 were 24.2 and 22.7 nmol/ mg, respectively. Jejunal digesta viscosity was linearly increased (P < 0.001) from 2.2 to 4.1 cP with increasing dietary CM levels. There were linear effects (P < 0.001) of CM1 and CM2 substitution on final weight, weight gain, feed intake, and G:F. The inclusion of CM1 and CM2 linearly decreased (P < 0.001) ileal digestibility of DM, energy, and IDE. The supplementation of CM1 and CM2 linearly decreased (P < 0.001) the retention of DM, nitrogen, and energy; ME, and ME, By regressing the CM1 and CM2-associated IDE intake in kilocalories against kilograms of CM1 and CM2 intake, the IDE regression equation was  $Y = -10 + 1,429 \times CM1 +$  $2,125 \text{ x CM2}, r^2 = 0.55$ , which indicates that IDE values were 1,429 kcal/kg of DM for CM1 and 2,125 kcal/kg of DM for CM2. The ME regression was Y = 5 + 882 x CM1 + 925 x CM2,  $r^2 =$ 0.54, which implies ME values of 882 kcal/kg of DM for CM1 and 925 kcal/kg of DM for CM2. ME<sub>p</sub> regression was Y = 2 + 795 x CM1 + 844 x CM2,  $r^2 = 0.52$ , which implies ME<sub>p</sub> values of 795 kcal/kg of DM for CM1 and 844 kcal/kg of DM for CM2. Based on these results, utilization of energy and nitrogen in CM by broiler chickens is low and the high viscosity observed in jejunal digesta as well as the total glucosinolate in CM may have contributed to the poor energy and nitrogen utilization.

**Key words:** *camelina meal, nitrogen corrected metabolizable energy, broiler* 



# O<sup>32</sup> Effect of Digestible Valine and Leucine Level of Broiler Grower Diet Based on Corn and Soybean Meal on Performance and Intestinal Development\*

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#### Abstract

This experiment was conducted to evaluate the effects of different levels of valine (Val) and leucine (Leu) in corn-soybean meal based broiler grower diets on performance, carcass parameters, bone and intestinal development. Two dietary digestible Leu (1.07, 1.50%) and three Val levels (0.64, 0.74, 0.84% 1) in a factorial arrangement of  $2\times3$  in 6 treatments (T1-T6), totally 6 treatments with 720 Ross 308 day-old broilers were used.

FCR in both dietary Leu levels significantly improved by increasing Val level (P<0.05) during grower phase. Significant Val×Leu interaction was found (P<0.05) for villus height, crypt depth.

Results of the present study showed the antagonism between Leu and Val so these two AAs must be taken into consideration when balancing low protein diets, besides function of Val in bone and gut development must be further considered.

Key Words: Broiler, Valin, Leucine, Performance, Jejunal morphology

#### Introduction

Feed cost represents 65% of the total live production cost for broiler chickens, on the other hand rate and efficiency of growth in broilers has increased through genetic selection compared to the broilers used from previous decades (Havenstein et al., 2003). A large portion of this cost involves meeting the amino acid (AA) requirements of the birds. By reducing the level of crude protein in the diet it is possible to achieve significant cost savings (Ruth McGill, 2009).

The modern broiler accretes more lean tissue per unit of feed consumption than broilers used in past research. Increasing dietary AA density enhances growth rate and meat yield of broilers (Corzo et al., 2010; Dozier and Payne, 2012). There has been an increasing trend to formulate diets on a digestible AA ratio concept in broiler production (Dozier, 2013). Digestible AA value and crystalline AA supplementation has allowed the poultry industry to reduce dietary crude protein (CP) to decrease excess amounts of AAs and the cost of rations (Kidd et al., 1996). So these dietary developments has been chanced broiler diet composition, order of limiting AA, and ratio among AA. The branched chain AAs (**BCAA**; leucine, isoleucine and valine) are the most hydrophobic AAs due to their unsubstituted aliphatic chain with a branched alkyl group (Patek, 2007). Studies have been shown an interaction among the BCAA. In the case of BCAA antagonism, nutritional need for Ile is increased due to increased catabolism of the BCAA in poultry (D'Mello, 1975). Besides the inclusion of supplemental valine (Val), leucine (Leu), and isoleucine (Ile) in diet may further reduce production costs without altering performance objectives (Corzo et al., 2007; Corzo et al., 2009; Corzo et al., 2010). So, the aim of the present trial was to evaluate the interaction between Val and Leu in low protein grower phase broiler diets on performance and jejunum histomorphology,

#### **Materials and Methods**

#### Birds, Hausing, Treatments, Feeds and Analysis

Two different Leu levels (SID Leu 1.07, 1.50%) and three levels of Val (SID Val 0.64, 0.74, 0.84%) in a 2×3 factorial arrangement were examined (table 1). All AAs used in the current experiment were provided from Evonik Industries, Istanbul-Turkey. Amino acids profile of each experimental diets were determined (Llames and Fontaine, 1994; Commission Directive, 1998). Starter (day 0-10) and grower (day 11-24) diets were based on corn, wheat and soybean meal. The study was carried out in Poultry House of Animal Science Department, Ankara University. Each treatment had 12 replicates with 10 chicks (totally 720 day-old male Ross 308 chicks) per 45×43 cm battery cages for 24 days. All treatments were given the basal starter diets (table 2) through 0-10 days. Treatment diets were introduced during grower period 11-24 days of age (table 2). Each cage was equipped with two inside nipple drinker and an outside galvanized feed trough. Birds had *ad libitum* access to water and mash feed throuhout the experiment.

Birds were weighed at the beginning of the experiment, at day 10 and 24 per each replicate. Feed consumption was also measured with the same periods. Feed conversion was calculated for 11-24 days using FI and WG for each replication. At day 24, 3 chickens per pen nearest to the average pen weight were selected for processing. A five centimetre long segment of each jejunum was dissected at midpoint toward to proximal duodenum, flushed with room temperature saline solution. Samples were cut into sections using a microtome at a thickness of  $5\mu$ m and then placed on a glass slide. Slices were then stained for variables measuring such as: villus height and width(Sakamoto et al., 2000; Solis de los Santos et al., 2005; Geier et al., 2011).

#### **Statistical Analysis**

The data for all response variables was analyzed as a completely randomized block design with 6 dietary treatments and 12 replicate blocks by using General ANOVA/MANOVA procedure of the SAS (2002), by analysis of variance or covariance. Main effects and interactions between the main affects were calculated in a  $2\times3$  factirial design including 2 levels of Leu and 3 levels of Val. Cage was the experimental unit for all analyses. When significant differences (P<0.05) among groups were found, means were separated using the Tukey HSD test. Mortality results were assessed by Chi-square test.

#### **Result and Discussion**

#### Performance

It is commonly agreed that greater performance in chicks can be achieved if the essential AA in low CP diets were equivalent to those needed in the higher CP diets and when the balance of AAs is maintained (Pinchasov et al., 1990; Çiftci and Ceylan, 2004). Several studies have addressed the Val needs of broilers in the growing phase (Thornton et al., 2006; Corzo et al., 2008; Tavernari et al., 2013).

In the current study two different levels of Leu had no significant effects on BW, BWG and FCR. Of course birds fed on diet containing 1.07% Leu had better FCR than 1.50% fed birds (P<0.05).



The effect of dietary SID Val levels on BW, BWG and FCR was significant (P<0.05) but it had no significant effect on FI. So, diets containing %0.74 and 0.84 Val had the highest BW and BWG and the best FCR compare to 0.64% Val (P<0.05). The Leu×Val interaction was found significant for FCR, and while 0.84% Val had the best FCR in low dietary Leu level(1.07%) (P<0.05), increasing dietary Val from 0.64% to 0.74% and 0.84% improved FCR significantly in diet containing 1.50% Leu, (table 3).

Ueda et al. (1981), indicated a decreasing trend of FI with increasing supplementation of dietary Leu. The reason for the toxic effect of excessive Leu on chicks fed with low protein diets is not well understood. In low protein diets, Leu may accelerate the degradation of other BCCAs, mainly in muscles through the stimulated activity of branched chain alpha-ketoacid dehydrogenase (Harper et al., 1984), which leads to the lower Val and Ile in tissues. Some studies (Jiang et al., 2005; Waldroup et al., 2005; Namroud et al., 2008) have reported impaired BWG and FCR when broilers were fed low protein AA-supplemented diets. A number of explanations for the discrepancies in performance between low CP experiments have been proposed, including differences in the level of CP and AA fortification, dietary ingredients utilized, chosen AA requirements (dietary designs), as well as bird age and strain (Corzo et al., 2005; Berres et al., 2010). Aftab et al. (2006) suggested that supplemented free AAs to low-CP might cause a sudden influx of AAs that increase the catabolism of AAs profile.

#### Jejunum Histomorphometric Analysis

The results belong to the effect of different dietary treatments on jejunal histomorphology are presented in table 4. The synthesis of protein, *i.e.* protein deposition in broiler chickens, is a process that requires a large amount of energy and is, to some extent, dependent on bird-related factors such as the development of gastrointestinal tract. The expansion of surface area that occurs with villus growth has been used to explain the increased absorptive capacity, whereas decreased villus height lowers the absorptive capability of the small intestine (Yamauchi et al., 1996). Hence, a higher villus height increases the intestinal surface area and consequently nutrient absorption (Soltan, 2009), and results in better performance. In the current study significant Val×Leu interaction was found (P<0.05) for villus height, crypt depth. Villus height in 1.07% dietary Leu level increased by increasing dietary Val from %0.64 to %0.84 (table 4; P<0.05). On the other hand crypt depth in low Leu diet (1.07%) decreased significantly by increasing Val level (P<0.05) and in diets containing high Leu level (1.50%) increased by increasing Val level (P<0.05).

#### Conclusion

In the current study the Leu×Val interaction were found significant, so that by increasing dietary Leu level the antagonism between Leu and Val might be happened and such antagonistic effect can affect the performance. It can be said that interaction between Leu and Val will be one of the most important aspects which needs high attention in formulating broilers' diets.

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Tractments	Starter phase,	Grower phase,			
Treatments	0-10d	11-	24d		
		SID Leu, %	SID Val, %		
T-1	Standard starter	1.07	0.64		
T-2	Standard starter	1.07	0.74		
T-3	Standard starter	1.07	0.84		
T-4	Standard starter	1.50	0.64		
T-5	Standard starter	1.50	0.74		
T-6	Standard starter	1.50	0.84		

#### Table 1. Experimental design of the trial

#### Table 2. Experimental basal diets, (%)

Feed ingredients	Starter diet	Grower
Corn	41.310	42.270
Soybean Meal	39.500	18.910
Wheat, Red	10.000	29.080
Sunflower oil	4.660	3.960
Dicalcium Phosphate	2.070	1.850
Limestone	1.210	1.180
Common Salt	0.300	0.100
NaHCO3	0.040	0.590
Vitamin premix	0.100	0.100
Mineral Premix	0.100	0.100
Choline Cl -60	0.020	0.070
DL-Methionine	0.340	0.370
L-Lysine HCl	0.210	0.580
L-threonine	0.100	0.260
L-Arg	-	0.290
L-Val	0.040	0.040
L-Ile	-	0.220
L-Trp	-	0.030
Total	100.00	100.00
Calculated chemical analysis <sup>1</sup>		
Metab. Energy, Kcal/kg	3030	3150
Protein	22.76	16.50
Calcium	1.03	0.92
Avail. Phos.	0.50	0.46
Leucine _dig	1.587	1.074
Valine _dig	0.950	0.640
Lysine _dig	1.270	1.100
Methionine _dig	0.638	0.582
_Met+Cys_dig	0.935	0.810

<sup>1</sup> dig= digestible


	SID	SID	BW, g	BWG, g	FI, g	FCR, g:g	Mortality,%
Treatments	Leu, %	Val, %					
T1	1.07	0.64	967.9±11.54	712.9±10.15	$1084.6 \pm 13.20$	1.522±0.0070 b	2.5±1.31
T2	1.07	0.74	984.9±12.73	727.0±13.16	$1100.9 \pm 15.86$	1.516±0.0124 b	1.67±1.12
Т3	1.07	0.84	986.5±11.28	730.8±10.22	$1087.5 \pm 12.43$	$1.489 \pm 0.0087 c$	0.83±0.83
T4	1.50	0.64	954.5±10.15	694.0±10.37	$1083.6{\pm}13.45$	1.562±0.0064 a	1.67±1.12
Т5	1.50	0.74	984.0±8.29	727.0±6.39	$1106.5 \pm 11.36$	1.522±0.0106 b	1.67±1.12
Т6	1.50	0.84	980.7±11.16	724.5±9.67	$1100.0{\pm}16.67$	1.518±0.0086 b	4.17±1.93
Main effects							
Leu							
	1.07		979.8±6.80	723.5±6.45	$1091.0 \pm 7.89$	$1.509 \pm 0.0059$	1.67±0.63
	1.50		973.0±6.00	715.2±5.63	$1096.7 \pm 8.02$	$1.534 \pm 0.0059$	$2.5 \pm 0.83$
Val							
		0.64	961.2±7.64 b	703.5±7.36 b	1084.1±9.22	1.542±0.0062 a	$2.08 \pm 0.85$
		0.74	984.4±7.43 a	727.0±7.15 a	1103.7±9.55	1.519±0.0080 b	1.67±0.78
		0.84	983.6±7.78 a	727.6±6.91 a	$1093.8 \pm 10.25$	1.504±0.0067 b	2.5±1.09
Р							
Leu			0.415	0.255	0.594	0.000	0.387
Val			0.038	0.012	0.328	0.000	0.777
$\text{Leu} \times \text{Val}$			0.820	0.568	0.875	0.035	0.179

**Table 3**. Effects of different levels of L-Valine and L- Leucine in low-crude protein diets during the grower phase on broiler chicks performance in grower phase (11-24 days).<sup>1</sup>

<sup>1</sup>Values are expressed as means  $\pm$ standard error mean of 12 replicates of 10 male broiler chickens; BW, body weight; BWG, bodyweight gain; FI, feed intake; FCR, feed conversion ratio (feed : gain) <sup>a-c</sup> Means within the same column without common superscripts are significantly different (P<0.05)

	SID Leu,	SID Val. %	Villus Length,	Crypt depth,
Treatments	0⁄0	51 <b>D</b> 7 any 70	μm	μm
T1	1.07	0.64	1168.2±26.90 c	182.8±5.84 b
T2	1.07	0.74	1184.7±28.27 c	171.7±3.55 b
Т3	1.07	0.84	1294.5±22.62 b	155.8±3.14 c
T4	1.50	0.64	1310.9±25.76 b	157.7±3.71 c
T5	1.50	0.74	1426.6±13.73 a	210.4±7.69 a
T6	1.50	0.84	1359.9±12.60 ab	197.7±1.95 a
Main effects				
Leu				
	1.07		1215.8±24.24	170.09±3.24
	1.50		1365.8±13.79	188.61±5.22
Val				
		0.64	1239.5±34.89 a	170.25±4.53 b
		0.74	1305.7±33.06 ab	191.04±6.24 a
		0.84	1327.2±14.86 b	176.75±5.38 b
Р				
Leu			0.0001	0.0001
Val			0.022	0.0003
$Leu \times Val$			0.028	0.0001

**Table 4**. Effects of L-Valine supplementations to low-crude protein diets with two different Leucine levels during the grower phase on jejenum villus parameters of broiler chicks at 24 days<sup>1</sup>

<sup>1</sup>Values are expressed as means  $\pm$ standard error mean of 6 replicates of 9 male broiler chickens. <sup>a-d</sup>Means within the same column without common superscripts are significantly different (P<0.05)

## O<sup>33</sup> The Effect of Different Levels of Lysine on Performance and Serum Chemistry in Sexed Broilers

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#### Abstract

A study was conducted to determine the effect of different levels of Lysine amino acid on the performance and serum chemistry in sexed broilers. The trial was conducted on 360 sexed birds, 3 treatments and 24 replicates. Three types of feeds were prepared i.e, Feed A, B & C. A diet contained 1.1% lysine throughout the experiment, diet B contained 1.04% lysine from day 1-21 and 0.95% lysine from day 22-42, diet C contained 1.08% diet from day 1-10, 0.99% from day 11 to 21 and 0.95% lysine from day 22 to 42. Duration of this experiment was 42 days. Statistical analysis of the data showed that dietary lysine improved the body weight, feed intake and FCR significantly (P<0.05). The significant difference was observed (P<0.05) in serum glucose, TP, Cholesterol and serum urea levels of broilers fed diets with different lysine regimes. Phase feeding of lysine resulted in improved performance and better serum chemistry. Key Words: Lysine, Feed Intake, FCR

#### Introduction

Broilers fed low protein diets, require higher lysine for maximum feed efficiency and weight gain (Labadan et al. 2001) because Lysine has very significant effect on growth. Since, Lysine amino acid exhibits specific effects on breast meat yield and composition of carcass (Schutte et al. 1995). Increasing lysine more than the recommendation of NRC (1994) has been reported with improved weight gain, improve breast meat yield and feed efficiency (Si et al. 2004).

Extensive work has been done to know accurate requirement of lysine by broiler chickens, which is recommended by NRC (Kidd et al. 1998). However, there is not still enough information about the differential requirement of male and female broilers. There is some suggestion of more lysine requirements of male broilers than female, because of their higher growth and body composition. Body weight and body confirmations, these two parameters are of much importance for measuring growth in the domestic chicken. A major role of lysine is assumed in breast meat development A broiler breast meat represents a large portion of the total carcass meat therefore, lysine is also found in high concentration in this portion of meat (Kerr et al. 1999).

Keeping in view the importance of lysine, present study was conducted to evaluate the effect of lysine supplementation on body measurements, blood chemistry and immune response in broilers.

#### **Materials and Methods**

The parameters such as body measurements were taken weekly, blood samples were takes twice in the experiment. i.e at day 21 and 42, and at the last, few birds were chosen for sensory evaluation of the breast and thigh meat after slaughtering. A total of 360 day-old Cobb sexed broilers were placed randomly in three groups i.e. A, B & C each having 15 birds each throughout 42 days of experiment. Male and female birds were kept separately. There were 8 replicates of



each group, having 4 replicates for each sex, in each having 15 birds. Birds in group A were fed lysine with level of 1.01% of diet throughout its life. In group B, birds were fed with lysine level of 1.04% of diet from 1-21 days, followed by 0.95% for the rest of 21 days. Similarly birds in group C were fed with lysine level 1.08% for the first 10 days, followed by 0.99% from 11-21 days and 0.95% from 22-42 days. Proper management practices were followed during the experiment. Birds were offered *ad-lib* feed and water during the experiment. All birds were offered the experimental diets in their respective cage replicates. At the end of experiment (  $42^{nd}$  day) one bird from each replicate was slaughtered to collect the slaughtering data. Weekly feed consumption was calculated. Chicks were weighed at the 1<sup>st</sup> day of their arrival and at the end of each week regularly to estimate weekly body weight gain. Total body weight gain was recorded at end of the trial. Data recorded for weight gain and feed intake were used to calculate weekly feed conversion Ratio. For the study of serum biochemistry parameters, 1 bird from each replicate was chosen. Blood was collected from the birds at 21st and 42nd day of experiment. Serum was analyzed for biochemical studies by Chemistry Analyzer at Clinical Pathology Lab. Department of pathology, University of Veterinary & Animal sciences, Lahore. Serum Glucose level was analyzed by method as described by Weismann et al. (1958) and Tietz (1995), serum cholesterol was analysed method as described by Roeschlau et al. (1974). Serum total protein level was analyzed by method (Biuret reagent) as described by Gornall et al. (1949) and serum urea level was determined by method as described by (Shephard et al. 1983)

Treatment	Duration (Days)	CP (%)	ME ( Kcal/kg)	Dig. Lysine (%)
A (Single Phase)	1-42	21	2900	1.01
B Phase 1	1-21	21	2900	1.04
Phase 2	22-42	18.4	3000	0.95
C Phase 1	1-10	21	2900	1.08
Phase 2	11-21	19	3000	0.99
Phase 3	22-42	18.4	3000	0.95

**Table 1-** Composition of Experimental Feed

#### **Statistical Analysis:**

The data recorded will be subjected to statistical analysis using Analysis of Variance Technique (Steel et al. 1997) in randomized complete block design (RCBD) with factorial DMR. (Duncan 1995).

#### Results

**Weight gain:** Weekly trend (Fig.2) as well as mean body weight gain showed significant differences (P > 0.05) with respect to different lysine phases at finisher stage (Table 2). Significantly weekly higher and mean body weight gain was observed in birds fed with 3 phase lysine diet as compared to those of 2 and 3 phase diets. However, sexes did not show significant difference in weekly as well a mean body weight gain at starter and finisher stage.

**Feed Intake (g):** Weekly trend as well as mean feed intake showed significant differences (P > 0.05) with respect to different lysine phases at finisher and starter stage (Table 3). Significantly

weekly higher and mean feed intake was observed in birds fed with 1 phase lysine diet as compared to those of 2 and 3 phase diets. However, sexes (Fig. 3) did not show significant difference in weekly as well a mean body weight gain at starter and finisher stage.

**Feed Conversion Ratio (g):** Weekly trend as well as mean feed conversion ratio showed significant differences (P > 0.05) with respect to different lysine phases at finisher and starter stage (Table 4). Significantly weekly higher and mean feed conversion ratio was observed in birds fed with 1 phase lysine diet as compared to those of 2 and 3 phase diets. However, sexes did not show significant difference in weekly as well a mean body weight gain at starter and finisher stage.

**Serum Glucose Level (mg/dL):** Significant differences (P<0.05) were observed among different lysine regimes in Serum Glucose levels at the age of 21<sup>st</sup> and 42<sup>nd</sup> day (Table 6). At 21<sup>st</sup> and 42<sup>nd</sup> day, higher value was observed in birds fed with 3 phase diet as compared to those of fed with 2 and 1 phase diets. As far as sexes were concerned, no significant difference was observed among glucose levels of male and female broilers at 21<sup>st</sup> and 42<sup>nd</sup> day of age. On overall interaction between sex and different lysine regimes maximum value for Glucose was observed in birds having 3 phase lysine regimes at the age of 21 and 42 days.

**Serum Cholesterol Level (mg/dL):** Significant differences (P<0.05) were observed among different lysine regimes in Serum Cholesterol levels at the age of 21 and 42 days. At 21<sup>st</sup> and 42<sup>nd</sup> day, maximum value was observed in birds fed with 3 phase followed by the bids fed with 2 and 1 phase diet. Similar trend was found at 42<sup>nd</sup> day (Table 5). As far as sexes were concerned no significant difference was observed in cholesterol level among sexes. On overall interaction between sex and different lysine regimes maximum value for cholesterol was observed in birds fed with 3 phase lysine regimes at the age of 21 and 42 days.

**Serum Total Protein (g/dL):** Significant differences (P<0.05) were observed among sex and different lysine regimes in Serum Total Protein levels at the age of 21 and 42 day (Table 7). At  $21^{st}$  day highest value was observed in birds fed with 3 phase diets followed by those of 2 and 1 phase diets As far as sexes were concerned, no significant difference was observed in serum total protein levels at  $21^{st}$  and  $42^{nd}$  day of age. On overall interaction between sex and different lysine regimes maximum value for Total Protein was observed in birds fed with 3 phase lysine regimes at the age of 21 and 42 days.

**Serum Urea (mg/dL):** Significant differences (P<0.05) were observed among sex and different lysine regimes in Serum Urea levels at the age of 21 and 42 day. At  $21^{st}$  day and  $42^{nd}$  day, highest value was observed in birds fed with 3 phase diet as compared to those of 2 and 1 phase diet (Table 8). Sexes were failed to show any significant difference in serum urea level at age of 21 and 42 days. On overall interaction between sex and different lysine regimes maximum value for Serum Urea was observed in birds fed with 3 phase lysine regimes at the age of 21 and 42 days.

#### Discussion

**Body Weight Gain (g):** Significantly (P < 0.05) higher weekly and mean body weight gain at starter and finisher stage may be due to that possibly that 3 phase lysine was optimum to meet the body requirement at different stages of the broilers as compared to 1 and 2 phase lysine. It may also be due to high lysine intake in starter phase which ultimately led to more body weight



gain. This result is supported by Labadan et al. (2011), who also reported that body weight of broilers was significantly higher when fed with diet higher in lysine at starter stage.

**Feed Intake (g):** Significantly (P < 0.05) higher fed intake was found in the birds fed with 1 phase lysine diet which contained low lysine as compared to others, which may be due to that this lysine level is not optimum for the growth of broiler. This result was opposite to the findings of Javad and Farshid (2011), who reported that increased feed intake was significantly highest in broilers fed with higher Lysine level in starter diet.

**Feed Conversion Ratio:** Best FCR was reported in the birds fed with 3 phase diet, which may be due to that 3 phase diet was optimum for the growth of broiler. As 3 phase diet contained 1.09% Lysine at starter and 0.95% in finisher stage, this result was similar to the finding of Lecleroq (1998) who also reported that body composition of birds was significantly affected by the amino acid Lysine when administered at higher level than those required, and showed improved FCR.

**Serum Glucose Level:** Higher serum glucose level in birds fed with 3 phase diet may be due to higher growth and metabolism of the birds. Similar findings were reported by Malomo et al. 2013, who reported that broilers had maximum serum glucose level (2.53 mmol/L) when fed with 091 % lysine diet as compared to other diets and the diet recommended by NRC, while reducing lysine further resulted in reduction of serum glucose level. i.e at diet with 0.55 % lysine glucose level was found 1.30 mmol/L. These results are also supported by Hernandez et al. 2012, who found that serum glucose level of male chicks was higher at all ages (266.6 mg/dL, 293.4 mg/dL and 280.6 mg/dL) as compared to female broiler chicks (226.9 mg/dL, 255.2 mg/dL and 264.5 mg/dL). It is also generally accepted that male birds have higher glucose levels than the female birds. (Scholtz et al. 2009, Peebles et al. 1997).

**Serum Cholesterol Level:** Studies have indicated hypercholesterolemia effect is unique to increase in dietary lysine level, and it was not observed by feeding any other amino acid in excess. Higher serum cholesterol level in the birds fed with 3 phase diet as compared to those of 2 and 3 phase diets may be due to that 3 phase lysine diet was optimum for the normal growth and metabolism. Similarly, Mehrdad et al. 2012. also reported that by increase in lysine levels of diet at day 21 and day 42 resulted in an increase of the serum cholesterol level of the broilers. He reported that increase in dietary lysine caused a linear increase in serum cholesterol level i.e 107.5 mg/dL at 1.1 % lysine diet, 136.5 mg/dL at 1.2 % lysine diet, 169 mg/dl at 1.3 % and 169.5 mg/dL at 1.4 % lysine diet. Dale et al. 2013 also reported that by increase in dietary lysine serum cholesterol level increases. Kokatnur. Et al. (1961) reported a marked hypercholesterolemia in chicks fed with excess dietary lysine.

**Serum Total Protein:** lysine is required in all species for protein synthesis and forms L-carnitine by combining with methionine; therefore, its deficit impairs protein biosynthesis (Ronald et al. 2007). Broilers fed low protein diets, require higher lysine for maximum feed efficiency and weight gain (Labadan *et al.* 2001) Lysine is second most limiting amino acid when broilers are fed with corn based diets. Lysine amino acid exhibits specific effects on breast meat yield and composition of carcass (Schutte *et al.* 1995). Highest serum protein level was recorded in the birds fed with 3 phase lysine diet, which determines that 3 phase diet is optimal and efficient for more growth and increase in body weight.. Mahdavi et al. (2012) also reported that serum total protein level was higher at all ages fed with higher dietary lysine , so they assumed that

increase in dietary lysine level causes increase in serum total protein level, which is opposite to the present results.

**Serum Urea:** Serum urea or blood urea nitrogen is indicator of normal liver function. Liver converts highly toxic ammonia into less toxic urea and uric acid which is then excreted from faeces of the birds. Highest value of urea in the birds fed with 3 phase diet suggests that 3 phase diet is optimum for the normal function of liver and hence normal growth of birds. Similar finding were reported by Powell et al. (2009) that increase in lysine level caused increase in serum urea and uric acid.

#### **Conclusion:**

So phase feeding is beneficial as compared to 1 phase diet or 2 phase diet. 3-phase diet contains less lysine level as compared to NRC requirements, so by feeding on less lysine level, better results can be obtained, when fed with low CP diet. This would result in decrease in feed cost, which is beneficial for poultry farmers.

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Table 2- Body Weight (	Jain (g) in se	xed broilers	s fed on di	fferent dieta	ry lysine pi	hases						
Age(Weeks)			Sta	rter					Fin	isher		
There are a contract of the second second second second second second second second second second second second	W Male	eek 1 Female	We Male	ek 2 Female	Wee Male	ek 3 Female	We Male	eek 4 Female	We Male	sek 5 Female	We <sup>r</sup> Male	ek 6 Female
LYSHIE FIIASES												
1-Phase	$167.29 \pm 9.14^{a}$	141.65± 7.37 <sup>b</sup>	429.49 ± 12.77 <sup>ab</sup>	$419.38 \pm 17.46^{\circ}$	841.56± 18.41 <sup>ab</sup>	819.70± 16.52 <sup>b</sup>	1327.43± 20.26 <sup>b</sup>	1301.67± 19.41 <sup>b</sup>	1907.75± 34.68 <sup>ab</sup>	$1844.84\pm 40.32^{b}$	2335.65± 64.91 <sup>ab</sup>	2249.43± 50.46 <sup>b</sup>
2-phase	153.34± 6.11 <sup>ab</sup>	147.67± 8.23 <sup>b</sup>	428.94 ± 19.28 ªb	$412.94 \pm 16.56$	829.01± 19.61 <sup>ab</sup>	813.01 15.37 <sup>b</sup>	1287.90± 20.67°	1271.12± 21.49 °	1880.76± 49.23°	1800.08± 30.78°	2288.32± 51.47°	2270.58± 48.90°
3-phase	161.46± 8.67ª	153.55± 6.27 <sup>ab</sup>	431.60 ± 10.88 ª	427.63± 18.76 <sup>ab</sup>	854.59± 18.29 <sup>a</sup>	$830.73 \pm 16.87^{ab}$	$1370.32\pm$ $18.43^{a}$	1348.13± 21.45 <sup>ab</sup>	$1930.36\pm 40.49^{a}$	1898.75± 41.76 <sup>ab</sup>	2427.20± 63.35ª	2447.76± 46.43 <sup>a</sup>
Table 3- Feed Intake (g)	) in sexed bro	oilers fed on	different	dietary lysir	ie phases							
Age(Weeks)			Sta	rter					Fini	isher		
	н	/eek 1	We	ek 2	We	ek 3	We	ek 4	Wee	k 5	Wee	<u> </u>
Lysine Phases	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1-Phase	$156.35\pm$ 7.98 <sup>ab</sup>	142.63 ±6.65 °	354.86± 23.67	340.03± 11.78	629.65± 9.03 <sup>b</sup>	615.32± 8.61°	888.19± 18.56 <sup>b</sup>	868.33± 15.52°	1138.87± 10.34	1126.92± 9.89	1330.67± 20.32 <sup>a</sup>	1318.45± 17.43 <sup>a</sup>
2-phase	159.46± 4.67ª	153.87 ± 3.25 <sup>b</sup>	358.17± 6.89	342.65± 5.98	653.76± 8.87 <sup>a</sup>	$633.10\pm 9.43^{ab}$	951.67± 13.65 <sup>a</sup>	$935.72 \pm 10.98^{ab}$	1177.47± 13.62	1157.67 ±14.19	1290.42± 18.32 <sup>ab</sup>	1280.89± 15.62 <sup>b</sup>
3-phase	$155.78\pm 4.89^{ab}$	$147.87 \pm 6.14^{\circ}$	329.48± 12.38	319.17± 11.43	623.43± 10.45 <sup>b</sup>	607.22± 6.67°	$869.13\pm 10.56^{b}$	859.76± 10.37°	1052.87± 13.69	1034.35± 14.67	1257.21ª± 21.21°	1235.83± 19.62°
Table 4- Feed Conversion	on Ratio (FC)	R) in sexed	broilers fé	ed on differe	ant dietary l	ysine phase	Sc					
Age(Weeks)				Starter						Finisher		
	1.0M	Week 1	Vielo	Veek 2 Eamolo	Mal	Week 3	olon M	Week 4	Nolo Wole	sek 5 Femele	Weel	c 6 2000-10
Lysine Phases	IMIAL	e remaie	INIAIC	remarc	INIAL			аю геша	ic Maic	remaie	Male	cillale
1-Phase	1.34	± 1.32±	1.28±	$1.24\pm$	1.53±	1.51	± 1.8	88± 1.80±	1.99±	1.93±	2.05± 1.97	.н. <sup>4</sup>
	0.23	<sup>a0</sup> 0.41 <sup>0</sup>	1.1	1.3	$0.20^{0}$	0.15	。 0.0	9° 1.2 °C	0.17	0.24	0.15 <sup>a</sup> 0.11	a0
2-phase	1.42	± 1.36±	1.5± 2.23	1.1±0	1.56±	1.52	н Вр	2ª 2.0±	2.09± 2.15	2.05± 2.05±	1.99± 1.97	<sup>+</sup> <sup>−</sup> <sup>−</sup>
2 where	0.74 72.0	11.0	CZ.U	40. 0 1 0 1	1.1.0	07.1		J. U.II	0.40	16.0	50.U 57.U	_
o-pnase	1.35: 0.12	± 1.29±0 b .04°	1.24± 0.08	1.24±∪ .07	±0.01 d 80.0	1.48 1.1 °	± 0.1	1± 1.6/± 4° 0.17°	$1.86 \pm 0.16$	1.82± 0.09	$1.83 \pm 1.81$ $0.11^{b}$ $0.1_{d}$	₩≏.

3rd INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI

136



Table 5- Serum Cholesterol Levels (mg/dl)	in Sexed Broilers Rea	ared On	Three Lysine I	Dietary
Regimens				

Age (Weeks)	D	ay 21	Day	42
Lysine Phases	Male	Female	Male	Female
1-Phase	$44.75 \pm 2.32^{d}$	$47.75 \pm 2.14$ <sup>cd</sup>	$72.25 \pm 1.31^{b}$	$71.50 \pm 2.72^{b}$
2-phase	$52.50 \pm 2.53$ bc	$55.25 \pm 1.65^{a}$	$79.25 \pm 0.63$ <sup>a</sup>	$80.75 \pm 1.80^{a}$
3-phase	$61.25 \pm 1.38^{a}$	$62.75 \pm 1.65^{a}$	$82.50 \pm 2.40^{a}$	$83.25 \pm 1.31^{a}$

Table 6-Serum	Glucose Lev	els (mg/dl) in	n Sexed	Broilers	Reared	On Thr	ee Lysine	Dietary
Regimens								

Age (Weeks)	Da	y 21	Day	y 42
Lysine Phases	Male	Female	Male	Female
1-Phase	$283.00 \pm 3.29^{\circ}$	$286.75 \pm 2.02^{\text{ bc}}$	$231.50 \pm 1.32^{a}$	$230.75 \pm 2.98^{a}$
2-phase	$295.50 \pm 0.30^{ab}$	$285.00 \pm 4.92^{\circ}$	$236.00 \pm 2.25^{b}$	$197.00 \pm 5.11^{b}$
3-phase	$306.25 \pm 2.06^{a}$	$309.25 \pm 1.25^{a}$	$252.75 \pm 4.91$ <sup>b</sup>	$263.75 \pm 6.60^{b}$

Table 7- Serum	Total Protein Levels	(g/dl) in Sexed	Broilers Reared (	On Three Lysine Dietary
Regimens				

Age (Weeks)	Daj	y 21	Day	y 42
Lysine Phases	Male	Female	Male	Female
1-Phase	$3.18 \pm 0.05$ <sup>c</sup>	$3.18 \pm 0.03$ <sup>c</sup>	$4.03 \pm 0.05$ <sup>b</sup>	$4.15 \pm 0.03^{b}$
2-phase	$3.48 \pm 0.05^{b}$	$3.50 \pm 0.04^{b}$	$4.50 \pm 0.04^{a}$	$4.55 \pm 0.03^{a}$
3-phase	$3.60 \pm 0.07^{b}$	$3.75 \pm 0.03^{a}$	$4.80 \pm 0.04^{a}$	$4.523 \pm 0.25^{a}$

 Table 8- Serum Urea Levels (mg/dl) in Sexed Broilers Reared on Three Lysine Dietary Regimens

Age (Weeks)	Day	v <b>21</b>	Day 42	2
Lysine Phases	Male	Female	Male	Female
1-Phase	$6.75 \pm 0.48^{\ d}$	$8.75 \pm 0.48$ <sup>c</sup>	$15.00 \pm 0.41$ <sup>b</sup>	$15.50 \pm 0.29^{b}$
2-phase	$10.75 \pm 0.48$ <sup>b</sup>	$11.00 \pm 0.41^{b}$	18.00±0.41 <sup>a</sup>	$18.00 \pm 0.41$ <sup>a</sup>
3-phase	$12.75 \pm 0.47^{a}$	$12.50 \pm 0.29^{a}$	$19.00 \pm 0.41^{a}$	$19.00 \pm 0.41^{a}$

## O<sup>34</sup> Effects of Genetic Selection on Meat Quality

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#### Summary

Genetic selection in broiler chickens has markedly increased production efficiency but has recently been associated with adverse effects on muscle and meat quality including pale, soft, exudative (PSE)-like meat; dark, firm, dry (DFD) meat; white stripping and "wooden" breast muscle. The underlying pathology of these conditions is similar and may have a common genetic aetiology. Substantial genetic version exists for related traits such as ultimate pH (pHu) and lightness (L\*) of breast muscle. Experimental selection experiments have been successful in changing the population mean for these traits. Taken together these results suggest that genetic selection would be highly effective in improving broiler muscle and meat quality. The advent of genomic selection based on DNA markers makes it possible to conduct genetic selection for muscle and meat quality without recourse to trait assessment on family members of selection candidates and could be conducted alongside genomic predictions for other traits. Recent research has shown that a substantial proportion of the phenotypic variation in muscle and meat quality traits can be explained by relatively few genetic markers and adds weight to the conclusion that genomic selection would be both feasible and successful. Undesirable consequences of genetic selection such as poor muscle and meat quality are inevitable and require a genetic solution (selection) when they arise.

#### Introduction

Genetic selection has been incredibly successful in increasing the feed efficiency of broiler chickens. This has been achieved by decreasing the age at a given slaughter weight (traditionally 2 kg), and by increasing the yield of edible muscle and relative proportion of breast meat in the carcass (Table 1). These changes have also been accompanied by undesirable changes that have affected the health, welfare and some aspects of muscle quality of the birds. Some examples such skeletal disorders, gait problems and feed restriction to maintain reproductive fitness have been the subject of extensive research. There are good reasons why deleterious consequences like these should appear and they will be summarised in the final section of this review. The main section of the paper will, however, address the current industry problem of muscle and meat quality.

Table 1.	Body weight,	carcass yield	and proportion	of breast	muscle in	different l	ines o	f broiler,
traditiona	al and layer cl	nickens at 6 w	eeks of age [1].					

Category	Lines, n	Weight, kg	Carcass yield, %	Breast muscle, % of carcass
Broiler	12	2.39	71.3	29.1
Traditional	13	0.59	61.4	18.2
Layer	12	0.54	60.8	17.5

#### Broiler muscle and meat quality

Pale, soft and exudative (PSE)-like breast muscle has been observed for many years and affects the appearance of the meat, water holding capacity, decreased tenderness and further processing



efficiency associated with an increased rate of post mortem pH decline [2, 3]. Broiler breast muscle in which the rate of pH decline is sub-optimal are characterised by a dark, firm and dry (DFD) muscle that is associated with atypical colour, flavour, texture and decreased shelf life [4, 5]. A physiological myopathy in broiler muscle was identified in the 1990s [6, 7] that was consistent with these changes. Mitchell and colleagues have characterised some of the physiological mechanisms underlying these changes [8, 9]. MacRae *et al.* [10] argued that as muscle fibre diameter was substantially larger in broiler than in layer muscle, and in broilers the fibre diameter in breast muscle was larger than in leg muscle, that the myopathy might be related to the lack of efficient diffusion of nutrients from the vascular system leading to subsequent muscle cell damage. Other evidence supports the conclusion that short term or long term prior stress increases the incidence respectively of both PSE-like and DFD broiler muscle [11].

More recently, there have been reports that a proportion of commercial broiler carcasses exhibit white striping, characterised by white parallel striations in the direction of the muscle fibres [12], or "wooden breast muscle" [13], conditions that lead, in some cases, to the condemnation of severely affected carcasses. In some cases, white striping has resulted in the rejection of a significant proportion of carcass after veterinary inspection at the slaughter plant: in one large study of 28,000 breast fillets randomly chosen from 56 broiler flocks, over 12% of samples were affected and showed similar characteristics to PSE meat [12].Pathological studies have shown that muscle abnormalities in samples affected by white striping are similar to those previously observed in birds at ambient temperatures that are exacerbated under conditions of heat stress [7] and lead to PSE-like muscle. These include degeneration, necrosis and regeneration of muscle fibres, angular myofibres, the presence of inflammatory cells, eosinophilic strands of collagen and thick cords of adipose tissue separating myofibres and muscle fascicles and [14, 15]. The breast muscle of broiler chickens is lighter and less red and yellow compared with traditional and layer lines (Table 2) However, scores for haemorrhages were higher and creatine kinase activity (CK), a marker of muscle cell damage, was elevated in the broiler chickens. Consistent with the elevated CK activity as an indicator of muscle cell damage, the concentrations of Na, K, Mg and Ca in plasma and muscle ash were higher in broilers compared with traditional and layer lines of chickens [16]. Furthermore both initial and final breast muscle pH were lower in broilers, and the decline in pH was greater, compared with the other two groups. Nevertheless the meat was more tender, requiring less shear force to cut the cooked meat, and taste panel scores were higher for texture, flavour and overall acceptability of meat from broilers compared with layers and traditional breeds [1, 16]. The differences in breast muscle function between broilers and layers (the latter presumably reflecting the wild type of breast muscle) remain if evaluated at the same age or body weight and are exacerbated by heat stress [17]. Taken together these results indicate that the normal physiological functions of broiler muscle cells are compromised leading to substantial decrements in the quality of broiler meat. This raises the question as to whether these differences are inherent in genetic selection for greater body weight and breast muscle yield or if they are merely circumstantial. This question can be answered at least partly by determining genetic parameters (heritabilities and genetic correlations) and by the results of selection experiments.

	2		,	2	
Trait	$t^{1}$	Broiler	Layer	Traditional	SED
Initial pH, pHi	0.34	6.09	6.16	6.16	0.017***
Ultimate pH, pHu	0.68	5.69	5.87	5.81	0.034***
Lightness, L*	0.37	55.9	53.0	52.0	0.67***
Redness, a*	0.25	2.79	3.50	3.98	0.27***
Yellowness, b*	0.43	2.74	4.04	3.72	0.28***
Haemorrhage score	0.61	3.74	2.04	2.08	0.18***
Creatine kinase, ln( IU/l)	0.94	$6.69(805)^2$	5.53(210)	5.35(205)	0.078***

Table 2. Breast muscle qualit	y of 37 lines or breed	s of broiler, traditiona	and layer chickens	[1, 16]	].
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<sup>1</sup> Intraclass correlation (between line variation divided by the total phenotypic variation). <sup>2</sup> (Backtransformed).\*\*\* P < 0.001.

#### Genetic aspects of muscle and meat quality

Observations based on general breed differences (Table 2) and specific comparisons [17] strongly suggest that a decrease in muscle and meat quality has accompanied genetic section for broiler traits, implying that a negative genetic correlation exists between broiler traits and muscle and meat quality. There have been several reports in the literature of the heritability of muscle and meat quality traits in recent years and these are summarised in Table 3. In general, heritability estimates are positive and moderately high (averaging 0.41) and genetic correlations are favourable or low for common quality traits measured on broiler breast muscle (Table 3). This evidence is consistent with the results from a multi-strain experiment. (A multi-strain experiment [18, 19] estimates the total genetic variation as the intraclass correlation defined as the between breed and/or line variation divided by the sum of the between and within breed or line variation.) The intraclass correlation for breast muscle quality traits (Table 2) ranged from 0.25-0.61 and for CK was 0.94. Taken together these results indicate that there is substantial genetic variation within lines that could be exploited to improve muscle and meat quality.

#### Genetic selection for broiler traits and muscle and meat quality

Long term selection for broiler traits (increased body weight and breast muscle yield with reduced abdominal fat) for 13 generations was associated with slightly higher pH in the selected compared with the control line [25]. Drip loss was significantly lower and breast muscle was paler (lower redness, a\* and yellowness, b\*) in the selected birds. However, the conclusion that genetic selection can lead to improved muscle and meat quality is supported by the results of two recent selection experiments for muscle quality.

Table 3 Mean herital	bility and gener	tic correlations	of muscle	and meat	quality t	raits in	broiler
chickens <sup>1</sup> .							

Trait	Herital	ritability			Genetic correlation			
			Breast yield		pHu		L*	
-	Mean	$N^2$	Mean	Ν	Mean	Ν	Mean	Ν
Initial pH, pHi	0.30	4	0.08	3	-0.04	2	-0.09	4
Ultimate pH, pHu	0.45	7	0.10	5	-	-	-0.64	4
Lightness, L*	0.49	9	-0.15	4	-0.67	6	-	-
Redness, a*	0.41	9	-0.27	4	0.03	6	-0.43	6
Yellowness, b*	0.39	9	-0.26	4	-0.27	6	0.34	6

<sup>1</sup>Data from [11, 20-25]. <sup>2</sup> Number of estimates.

Alnahhas *et al.*[20] selected two lines of broiler chickens for high or low final breast muscle pH. After 5 generations of selection there was a difference of 0.42 pH units between the lines. Breast



muscle colour in the high line was lighter (L\*, -5 units) and paler (a\*, -0.22 units; b\*, -1.53 units) and drip loss was greater (-1.6%) than in the low line. Cooking yield, cooking loss and toughness were all worse in the low compared with the high pH line. Genetic selection produced a difference between the lines of 0.9 genetic standard deviations for breast meat yield but body weight was similar in both lines.

Divergent selection for muscle lightness (L\*) was investigated by Harford *et al.*[11] who compared the selected lines and a random bred control line after 8 generations of selection. Mean L\* for the high and low lines was 53.91 and 46.86 respectively and the random bred control was intermediate (49.70). Selection for increased L\* also increased yellowness (b\*) and an increased rate of pH decline post slaughter whereas selection for decreased L\* increase redness (a\*) and decreased the rate of pH decline. Drip loss was greater in the high line compared with both the low and random bred lines which were not different. The changes associated with genetic selection for high L\* are typical of PSE-like meat and suggests that genetic selection would be effective in improving muscle quality in pedigree flocks. No results were presented for body weight differences between the lines.

The post mortem measurement of pHu or L\* is relatively simple and inexpensive and genetic correlations with traits related to consumer experience and further processing characteristics are generally high and favourable indicating that they could serve as useful selection criteria. The genetic correlation between drip loss with pHu, for example, has been reported as -0.68 [21]; -0.69 [22]; -0.83 [23]; -0.89 [24]; and -0.80 for the low pHu line of Alnahhas et al.[20]. Similarly, genetic correlations between drip loss and L\*, expect for the report by Gaya et al. [22], were of opposite sign and similar magnitude as those for pHu.

#### Genomic selection

Genetic selection for carcass traits like pHu and L\* are only possible on measurements of sibs which are costly to make unless other traits are also recorded such as breast meat yield are recorded at the same time. However, indirect selection on the basis of DNA markers is a technology that has recently become available and has the potential to facilitate considerable progress in improving the muscle and meat quality of broilers. Publication of the details of a dense SNP (Single Nucleotide Polymorphism) chip in 2013 [26], combined with the associated high-throughput SNP genotyping technology, has made this goal technically feasible and most of the poultry breeding companies have invested in it. Chen *et al.* [27] evaluated whole genome selection using real data and showed that the inclusion of genome information increased the accuracy of estimated breeding values for body weight, breast muscle area and leg score respectively by 20, 18 and 32 % compared with trait heritabilities of 0.20, 0.30 and 0.11, illustrating the potential benefits of genomic selection.

Evidence for a relationship between SNPs and broiler muscle and meat quality traits was obtained in a two stage experiment in the author's laboratory, demonstrating the potential of genomic section for improving broiler muscle and meat traits. In the first stage a microarray experiment was conducted to compare gene expression levels in broiler and layer chickens subjected to control (21°C, 50% RH) or heat stress (32°C, 75% RH) for 2 hours in a climate chamber. (A microarray experiment identifies genes that are up- or down-regulated in birds on one treatment compared with another e.g. broiler vs layer). Zahoor and colleagues identified 25 genes that showed a statistically significant interaction between broilers and layers that, on

the basis of published information, could affect muscle and meat quality related traits or the expression of downstream genes that affected these traits. In the second stage, a total of 100 SNPs in these genes were identified from the published genome sequence. SNP assays (primers) were prepared and the DNAs of 134 birds from a multi-strain experiment were genotyped with these SNPs. The data were then analysed by a rigorous multiple regression procedure and a number of significant relationships were detected between SNPs present in different genes and muscle and meat quality traits: 4-7 genes and 5-7 SNPs were associated with 26-55% of the phenotypic variation for bodyweight, CK, pHu and L\*. These results suggest that there are genes or mutations with substantial effects of muscle and meat quality and demonstrate that genetic selection based on DNA markers could result in substantial improvement in muscle and meat quality traits.

#### Deleterious consequences of genetic selection

Why have undesirable consequences such as poor muscle and meat quality appeared in genetic selection programmes for improved feed efficiency and carcass yield? In fact genetic theory predicts that consequences like this are an inevitable result of genetic selection [28]. Firstly, artificial selection for production traits and natural selection for adaptation to the production environment result in "selection sweeps" that increase the frequencies of rare recessive alleles (gene variants) that affect fitness (e.g. muscle function) negatively when in the homozygous state. Secondly, the effective (genetic) size of pedigree flocks is relatively small leading to "genetic drift" adding to this process and increasing homozygosity. Thirdly, genetic correlations between two selected traits will eventually become negative as genes and alleles responsible for positive correlations become fixed, leaving only those causing a negative genetic relationship between the traits. It is highly likely that these processes have been occurring in genetic selection for broiler traits and it is imperative that pedigree breeders add muscle and meat quality traits to their selection programmes because of their fundamental importance to the broiler industry..

#### Conclusions

Genetic selection for broiler traits was resulted in compromised muscle function and undesirable meat quality attributes. This outcome is inevitable and not unexpected. However, substantial additive genetic variation exists that will facilitate genetic selection for improved muscle and meat quality and in general genetic correlations with production traits are favourable or low. Genomic selection based on DNA markers is now technically feasible and could be adopted to achieve this objective in a cost effective and efficient manner.

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## O<sup>35</sup> Myths, Distortions and Lies About Poultry Meat and Production Systems

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#### Abstract

One of the most important challange of broiler industry is fears of consumers related to lies from media on poultry meat. Many of these fears are based on information or deliberate statements comes from incompetent and ignorant specialists with poultry, many of from medicine science and, media news with using these lies. Consumer perceptions are mainly focused on antibiotics, hormones, GMO, taste problems, fast growing, some chemicals, village hens and health of humans because of the mentioned issues. Most of these claims are unreal. Modern (conventional) poultry meat production is fully based on scientific methods, laws and regulations set by goverments which focused on animal health and food safety and, the production from farm to fork are continuously observed and inspected by government bodies to protect the consumers. Although some small differences among countries for current production model, similar production practices are apllied all developed countries in poultry industry. Similar broiler meat breeds are used all around the world fort his purpose. These breeds are all obtained by artificial breeding and selection methods from a cross of a male of a naturally double-breasted Cornish strain and a female of a tall, large-boned strain of white Plymouth Rocks. Turkish poultry meat industry which also used same cross breed hybrid birds selected artificially for fast growing and more breast meat for many years have been improved significantly and reached to big producer category in a global scale. Poultry meat industry will be expected to be the largest meat sector near future as a healthy, cheap and valuable protein source for humans. So limiting the broiler meat, consumed more than 30 kg per capita in developed countries, with mostly unreal news needs to be stopped in our country.

Key words: poultry meat, consumer concern, media, hormone, antibiotics, gmo

### O<sup>36</sup> The False Perceptions About Chicken Slaughtering by Consumers and Media in Turkey

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#### Abstract

Today, the poultry sector of the most advanced part of the agricultural production under the influence of the field to be related to consumption has become one of the production. Genetics, breeding, food industry, slaughterhouses and advanced processing equipment industry, eggs and egg products industry, incubators, health care, pharmaceutical and vaccine industry, marketing, improvements in housing and husbandry systems has created a fast-growing production.

Breeding, feeding, breeding techniques and has been an increase in the efficiency with advances in disease control. 2-2.5 kg slaughter weight in broilers 6 weeks to the contact with high survival benefit from 1.6-1.8 feed and hybrids have been developed. Worldwide; Between 1970 and 2014, production of poultry meat, beef, pork and sheep meat increased faster than production. Poultry meat production in a continued growth trend, despite the economic developments and emerging issues in the field of health in chickens has not been a change in output growth. The future is expected to continue this trend. In the year 2014 2010, 2011, 2012, and the world production of poultry meat production by 1,5-1,8% increase compared to 2013 reached 103-105 million tons.

In the world in poultry production; In 1970, 22 in 1980, 22 in 1990, 18 in 2000, 22 in 2005, engaged in the production in 2012 with a serious increase in production in Turkey in 2013 and reached the first 8-10 ranks pickup point in 2014.

The high cost of investment in the chicken slaughterhouse meat production requires integration with the production system. This makes product standards healing effect. However, animal welfare directives, consumer demand, social pressures, the impact of alternative production systems, demands for compliance with the belief in the sector can influence the consumption of chicken meat. Criticism with false information of products without restriction in the media "no religion and belief system" is not prohibited by the consumption of chicken meat may create negative results. Broiler production level that is effective in 95% of chicken meat consumption is mainly affected areas in this regard. In this paper, slaughter the chicken in our country during the pre-cut regarding applications and stunning, bleeding and killing, and feather removal that occur in a single-stage evaluation is made in terms of halal standards.



## O<sup>37</sup> Developments and Future Trends in Poultry Meat Processing

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#### Abstract

Today large dedicated poultry processing plants can be found all over the world. Plants are located in areas where poultry (chickens, turkeys, ducks) are grown, and usually service growing farms within a distance of up to 500 km. Over the past few decades a lot of mechanization and automation have been implemented and resulted in more efficient lines and increasing line speed from 3,000 broilers per hour in 1970 to 14,000 today.

This presentation will focus on some of the major developments that have allowed moving from manual to automatic procedures. These developments include live bird loading and unloading, scalding (hot water, steam), automated sorting (computerized image analysis), electrical stimulation, chilling, automated cut up, and portioning. Requirements for improving sanitation standards and pathogen reduction will also be highlighted.

#### Introduction

The increased demand for fresh and further processed poultry has resulted in the introduction of more mechanization and automation in all steps involved in raising and processing poultry around the world. These advances have also led to a significant increase in line speed (Table 1). On farm mechanization includes items such as automated feeding, watering and temperature control. When it comes to harvesting live poultry, prior to shipping to a meat processing plant, more machinery is currently used to help employees. This ranges from using a fork lift to get small crates / large modules in and out of the barn, to employing mechanical harvesters that eliminate the manual catching of birds. As this operation can have a significant effect on the level of downgrading at the processing plant, it will be used as the first example of advances made in automating poultry processing steps. The other major steps involved in primary processing are shown in Figure 1. On arrival to the plant, unloading of the birds from the crates has also been improved and automated to different degrees with an emphasis on minimizing bruising and quality defects. Also at this step a fully automated option is currently available. Scalding is done after slaughter to help loosen the feathers. The traditional process consists of immersion in a hot water bath (e.g., 3 min at 50°C), however today a new procedure employs steam. The process is known to help conserve a significant amount of water (some reports suggest 70%), energy, and potentially reduce microbial problems. During the past year a few Turkish companies have installed the system and are already benefiting from the process.

Electrical stimulation is another procedure which was introduced into large commercial plants 10-15 years ago. The goal of this step is to trigger muscle contraction and speed up the rigor process. This can help in shortening the waiting time needed to start deboning. The next step is primary processing is evisceration which in large plants is commonly performed by fully automated machines that are set to make all the necessary cuts. Also in this area there have been quite a few advancements that have resulted in high precision equipment that can be adjusted to different size birds while minimizing cross contamination. Following this step,

the birds are washed/rinsed and chilled to comply with food safety regulations. The common methods employed today include water immersion, air chilling and spray chilling. Large scale counterflow water chillers are employed by the industry to both reduce the temperature and also wash off microorganisms. Air chilling operations are also popular today in Europe, and include large tunnels with an extensive overhead rail system. At the end, the birds are either packed whole or cut up into portions to accommodate market's demand. Fairly sophisticated cutting equipment has been developed over the past two decades and today allows replacing / competing with manual deboning.

#### Loading and Unloading

Most poultry (chickens, turkeys, ducks) around the world are gathered and manually loaded into crates/modules. The process requires quite a few people when dealing with a large barn. Over the past three decades, various attempts have been made to mechanize the process and today a few viable options are available, with the most advanced system being able to operate inside the barn. The system includes a small tractor with a wide front end that collects broilers (different configurations available from different manufacturers) and placed then on a conveyer belt that moves to crates at the back of the tractor. Computer controls can be used to place a pre-determined number of birds in each crate.

The system requires 2-3 skilled employees and can replace 5-7 people on the loading crew. In addition, it can reduce some of the problems associated with manual loading (e.g., fatigue, potentially rough handling of broilers). The mechanized system requires a large capital investment but the payoff time, especially where labor cost is high, can be a couple of years.

At the processing plant birds are commonly unloaded by people standing along the truck (e.g., fixed cages) or along a conveyer belt if the cages are removed from the truck.

Tilting cages/modules, which have side doors, is also employed by some plants where the birds are then placed on a moving conveyor belt. This process is classified as a semi-automated procedure and the birds are then placed by employees on a moving shackle line.

If controlled atmosphere stunning (CAS) is to be used, the birds can be moved to a special area and are not placed on the shackle line while they are conscious. In all case, special care should be used when crates/modules are emptied by people or mechanically tilted to prevent damage / bruising to the birds.

#### Scalding

This process is employed to loosen the feathers from their follicles and make the de-feathering process easier and more efficient. In the traditional scalding process, birds are immersed in hot water (temperature can range from 50 to 62°C for short time of 1 to 3 min). The time and temperature depend on the type of bird, age, and further chilling method (water, air). In large processing plants this is done in a long tank(s) where the birds are moved along the line while the water is agitated to achieve good penetration in between the feathers. A new development consists of steam scalding where the birds are moved through a steam chamber while forced air movement is helping to get the steam close to the feather follicles. This process saves a substantial amount of water (some claim 70%) and energy to heat up all that water. The process also helps control the number of microorganisms on the carcass since it does not use a common



bath. Overall conditions in commercial scalders range from mild or the so called soft scalding  $(50 - 53^{\circ}C \text{ for } 2 - 3 \text{ min})$ , to sub scalding  $(54 - 58^{\circ}C \text{ for } 1 - 2 \text{ min})$ ; used for mature birds where feathers are more tightly attached) to the so-called hard scalding  $(60 - 62^{\circ}C \text{ for } 1 - 1.5 \text{ min})$ ; mainly used for waterfowl). Higher scalding temperatures are better for loosening feathers, but are also harsher on the skin and will result in the removal of the outer skin layer. As mentioned above, controlling the microflora at this stage is very important. Overall the relatively high water temperature (>50 C) is helping to inactivate certain groups of microorganisms. Another important improvement has been the introduction of counter-flow design during water scalding (clean hot water introduced at the exit end of the tank). In addition, some processors are also using several smaller tanks where the last one is the cleanest (successive washing effect).

#### **De-feathering**

At this step the feathers are pulled off / rubbed off the carcass by flexible rubber fingers. During the operation, the birds, on the shackle line, pass in between numerous discs/drums with fingers. The fingers are made of rubber and also contain certain lubricants to control their elasticity, which is important when removing feathers from different areas of the carcass. Today this is a completely automated process, in large poultry plants, and saves quite a lot of manual labour. At the end of the process the small pinfeathers on broilers carcasses are removed by a clean burning gas, so no off flavours are introduced. When waterfowl is processed and more pinfeathers are present, the birds are dipped in hot wax which is later on cooled down and peeled off. The wax can be re-melted, filtered, and reused. The plucked birds are then rinsed to remove any debris remaining.

#### **Electrical Stimulation**

This is an optional step in which a current is passed through the carcass in order to cause muscle contraction. This helps to use up the energy reserves in the muscle and shorten the rigor mortis process. This is turn can allow earlier meat deboning/harvesting from the carcass. Applying electrical stimulation is very important if early deboning of tender meat is required. In that case, deboning can start 3.5 hr after slaughter as compared to 6-8 hr without electrical stimulation. The process was originally developed in the 1960s for red meat animals (e.g., sheep meat exported from New Zealand) and has only started to appear in poultry processing plants 10-15 years ago. Today a number of large poultry processing plants are using this step while keeping the birds on a moving shackle line until deboning time. If deboning time is done the next day, the birds are removed from the line, kept in large containers, and rehung on the processing line prior to deboning. It should be noted that early deboning, without electrical stimulation, will result in significant muscle contraction and tough meat.

#### Evisceration

Developing semi-fully automated evisceration systems has been one of the first steps in mechanizing primary processing of birds in the early 1970s (Table 1). Prior to that a large workforce was needed to manually eviscerate poultry. Today most large plants have automated equipment that is used to make the necessary opening cuts that allow inserting a scoop-like device to remove the viscera. Over the years, there have been numerous advances in mechanizing the process and dozens of patents have been filled. The fully automated process can handle today up to 14,000 birds/hr. Special care should be taken not to damage the viscera while making the opening cuts, releasing the vent, and while withdrawing the viscera. Machine adjustment to deal with variation in flock size is also very important. In the event that a gut spill occurs, the

contaminated area might have to be trimmed or the whole bird condemned, depending on the specific local regulations. Before the birds leave the evisceration area they are washed / rinsed to remove any loose tissue / debris. An inside/outside bird wash station is commonly used for this purpose. The outside wash can be done by stationary sprays located along the processing line where the bird is washed / rinsed from top to the bottom. An inside wash/rinse can include simple spray nozzles or a more sophisticated retractable shaft that goes inside the cavity and sprays water at high pressure.

#### **Inspection and Sorting (Image Analysis)**

The birds with the viscera attached or detached (i.e., placed on a parallel moving line) are inspected by designated personnel. In some countries it is required that each bird be visually inspected by a qualified veterinarian, while in other countries a whole flock inspection is required. It should be pointed out that the rate of inspection by each individual inspector is limited to a reasonable number that can be efficiently inspected. During individual bird inspection, the entire carcass and internal organs should be visible to the inspector (e.g., a mirror can be placed on the backside of the line). The inspection station should be adequately lit so no shadows or under lighting interfere with the process. The station should also be equipped with a source of running water, a rack for positioning suspected birds and a bin for condemned birds.

Image analysis systems are also becoming popular in helping in looking for defects. High speed cameras are used in some fast production lines, to capture and digitize the image of every bird and compare it to a standard. Those systems are used today mainly for grading and not so much for inspection purposes. However, it is expected that in the future, regulations will be modified and also allow those systems to assist in actual inspection. The advantages of a modern system are high speed, high degree of accuracy, consistency, and the option of learning / adding more references at any given time (using fuzzy logic).

#### Chilling

Cooling down the meat is essential in controlling microbial growth. As the broiler's body temperature of around 40°C is ideal for all microorganism to grow, cooling helps to substantially reduce the rate of microorganism multiplication, spoilage, and risk of food borne diseases. The most common cooling methods include water, air, and spray chilling. Water chilling is currently the most popular method used around the world. Birds are immersed in long chilling tanks while the water is kept cold by a refrigeration system or use of ice so (i.e., important to maintain a temperature gradient between the meat and water to achieve a fast and efficient process). As with the scalder, most chillers today employ a counter flow design where the coldest water is introduced at the exit point of the birds. This also helps to get a more efficient process and lower microbial count at the end. It is often seen that the birds coming out of the water chiller carry lower microbial load than those entering the tank. Using a post-chiller is another popular improvement used today, especially where anti-microbial agents (chlorine, peracetic acid) can be used. There are also certain regulations about the amount of overflow water required for each bird going through the chilling tank (e.g., 2.5 L for each bird weighing up to 2.5 kg).

Cold air chilling is currently more popular in Europe where water price is higher (fresh water and waste water treatment). In such an operation the birds are moved through a special tunnel in which air flow (derived by large fans) is used to assist in removing heat from the meat. In most large operations the birds are placed on a moving shackle line which circulates through the



tunnels. To prevent too much moisture losses, the birds can be sprayed at certain points along the line (usually the turning point of the shackle line). Air chilling usually takes more time than water chilling, but the resulting product is drier, and more appealing to certain consumers.

Spray chilling is a hybrid between water and air chilling where cold water is constantly sprayed over the carcasses while they are moving along the line. Water pick up is lower than during water chill but higher than air chilling.

#### Portioning

Cutting and portioning can be done manually in small scale operations or assisted by machines in large processing plants. Mechanized equipment can be designed to cut the meat at predetermined locations and today (e.g., used for 'nine cuts' used by the fast food industry). The new generation of machines is often equipped with physical sensors, cameras and robotic arms to achieve gentle removal of the meat from certain poultry parts. The sharp decline in electronic cost (computers, cameras) has allowed the introduction of relatively inexpensive sensors and data processing devices to be used in various meat processing plants. There have been several generations of deboners for breast meat filleting, thigh meat deboning, whole leg meat deboning, etc. The challenges include obtaining high yield with minimal damage to the meat and/or bones.

#### Summary

The poultry industry has made significant progress over the past half a century in increasing line speed (Figure 1), efficiency and productivity while bird size has kept on increasing. Those tremendous changes have been the result of our accumulated knowledge in engineering, muscle biology, nutrition, computer science, and microbiology. It is expected that more changes will help enhance the process and also result in further improvements of food safety issues around the world.

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Table 1	Increase	in broiler	processing	line	speed	(bird/hr)	from	1970	to 201:	5 and	signific	ant
developn	nents. Ba	sed on Ba	rbut (2014)									

Year	Line Speed	Equipment Development
1970	3,000	Mostly manual operation
1975	4,500	Semi-automatic evisceration
1980	8,000	Fully automatic evisceration
1990	9,000	Giblet harvesting
2000	10,500	Cut up machine together with inline chilling
2010	12,000	Automated unloading and stunning
2015	14,000	More computer control

Figure 1 Typical sequence of operations in a poultry processing plant.

Transportation and Receiving  $\downarrow$ Unloading  $\downarrow$ Stunning (electrical, gas)  $\downarrow$ Bleeding  $\downarrow$ Scalding (water, steam)  $\downarrow$ De feathering  $\downarrow$ Washing / Rinsing  $\downarrow$ Removing Oil Gland, Feet and Rehanging  $\downarrow$ Viscera Removal  $\downarrow$ Inspection (mandatory) and Sorting  $\downarrow$ **Giblet Harvesting**  $\downarrow$ Lung, Crop, and Head Removal  $\downarrow$ Washing / Rinsing  $\downarrow$ Chilling (air, water)  $\downarrow$ Grading  $\downarrow$ Portioning and Packaging



## O<sup>38</sup> Pastırma Flavored Chips Production From Chicken Meat

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#### Abstract

Todays consumers' preference towards the healthy foods make meat technologists to develop new products and to improve the quality of the existed ones. Chicken meat is an important source of high quality protein with a good number of essential amino acids and its easily digestible characteristics. Therefore, chicken meat might be a very important source of future's new food product developments in our country where deficiency of animal origin protein in the diet is observed. The objective of this study was to develop the pastirma flavored chips produced with chicken meat. Furthermore, one of the aims in the presents study was to introduce a new food product with traditional *Turkish tastes* to the market which would also meet today's consumer's desires. Pastirma flavored chips were produced by mixing chicken breast meat, flour, spices and cemen to make a dough in a home style blender. The dough was then shaped as chips form and dried using a pilot scale tray drier. Chicken chips had 19% moisture. The CIE lightness (L\*), redness (a\*) and yellowness (b\*) values were 49.4, 15.7 and 34.9, respectively after drying. In sensory analysis conducted by evaluating appearance, taste, texture, color, seasoning level and general acceptability, the chips possessed low texture scores which could be attributed to the insufficient crispness in the final product. Future research should focus on improvement of the texture characteristics of the pastirma flavored chicken chips.

Keywords: Chicken meat, pastirma, chips, new product

3<sup>rd</sup> INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

## O<sup>39</sup> Incidence of *C. perfringens* in Chicken Neck Samples Taken From Slaughterhouse, Molecular Characterization and Definition of It's Antibiotic Resistance Profiles

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#### Abstract

The objectives of this study were, to determine the incidence of *C. perfringens* in 180 chicken neck samples, to detect *cpa* gene by PCR for the verification of the isolates and to identify toxin genes by multiplex PCR, to evaluate the seasonal distribution of *C. perfringens* prevalence, and to find out the antibiotic susceptibility profiles of the isolates. *C. perfringens* was isolated from 39 (21.6 %) of the 180 chicken meat samples by cultivation method. For the confirmation of isolates, PCR assay was perfromed. From 39 isolates *cpa* gene were determined in 35 (89.7 %) of them. None of the isolates possesed the toxin genes *cpb, cpb2, etx, iA* and *cpe*. During the winter (December, January, February) 13 and during the summer (June, July, August) 22 samples were found to be contaminated with *C.perfringens* so for the incidence of *C. perfringens* seasonal difference was not significant for chi square test. According to the disc diffusion test all of the isolates were found to be resistant at least one of the 9 antibiotics. 35 isolates was resistant to trimetoprim (100 %). In addition, high level of resistance was also determined against tetracycline (65,7 %) and gentamycin (62,8 %).



## O<sup>40</sup> Effects of Rosemary Oil on Chemical and Microbiologycal Features of Chicken Meatball

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#### Abstract

This study were conducted to examine effects of rosemary oil on chemical and microbiologycal features of chicken meatball. This aim chicken meat ball were divided three group as rosemary oil amount (K, B5 and B1 group) and rosemary oil were addition K group samples 0%, B5 group samples 0.5% and B1 group samples 1%. The meatball samples were analyzed sensory, microbiologycal (Total psychrotrophile bacteria, Enterobacteria, Mould-Yeast number) and chemical pH, thiobarbutiric number) on storage day0., 3., 5. and 7.. The microbiologycal analyzed were determined between B1 and B5 group not different. The sensory analyzed result of experimental samples were determined rosemary oil compatible with chicken meat.

Key words: chicken meat, meatball, rosemary oil.

### O<sup>41</sup> New Strategies to Improve Broiler Welfare

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#### Introduction

On 30 June 2010 new EU rules came into force across the EU (Council Directive 2007/43/EC) (EU 2007) protecting the welfare of broiler chickens. In the UK a meat chicken welfare 'trigger system' was introduced to fulfil the requirements of the EU Meat Chicken Directive (2007/43/EC), and has been operating in slaughterhouses since July 1st 2010. Post-mortem inspections carried out at the slaughterhouse are used to identify possible welfare problems on-farm, and a system runs in parallel with an exception reporting system that allows for immediate reporting and response when serious welfare infringements are observed.

In line with the requirements of the Directive, a number of animal based outcome parameters are used to identify possible on-farm welfare problems (Butterworth 2013a,b, 2011, 2010, 2007; Butterworth & Weeks 2010; Botreau et al 2007). Cumulative daily mortality rate and seven post-mortem conditions are monitored. The post-mortem conditions are: 1) Ascites/Oedema, 2) Cellulitis & Dermatitis, 3) Dead on arrival, 4) Emaciation, 5) Joint Lesions/Arthritis, 6) Septicaemia/Respiratory and 7) Total rejections 8) Cumulative Daily House Mortality (CDM), and 9) Foot Pad Score (FPD).

#### The system involves two processes:

**Process 1:** An alert to Animal and Plant Health Agency (APHA) is triggered if the rate of any of the post-mortem conditions is exceptionally high (defined as greater than 6 standard deviations above the mean.

**Process 2:** An alert to APHA is triggered if the CDM is unusually high (defined as greater than 3 standard deviations above the mean = 7.37%) and, additionally, the level of three or more of the post-mortem conditions is high (defined as above the mean, see Table 1).



Post-mortem condition	Process 1 trigger level	Process 2 trigger
	(%)	level (%)
Ascites/Oedema	2.02	0.21
Cellulitis & Dermatitis	3.00	0.20
Dead on Arrival	1.51	0.12
Emaciation	0.67	0.04
Joint lesions	0.43	0.02
Respiratory problems	9.28	0.49
Total rejections	11.76	1.11
Cumulative Daily Mortality	11.85	NA
FPD score*	167	60

Table 1 Process 1 and Process 2 trigger level measures, their thresholds, and notes	s on
calculation of trigger 2 (which combines a number of measures) and on FPD (Swea	dish
Foot Pad) score calculation.	

*Process 1. AHVLA will be alerted if the level of a post-mortem condition is exceptionally high (exceeds mean* + 6SD).

Process 2. AHVLA will be alerted if the Cumulative daily mortality rate is unusually high (exceeds mean + 3SD = 7.37%) and, additionally, the rate of three or more post-mortem conditions is high (exceeds the mean).

\* The FPD score is not a percentage but is a score of the severity and extent of lesions (between 0 and 200) based on scoring 100 feet.

#### How commonly are triggers raised?

When a process 1 or process 2 trigger level is exceeded, this is automatically registered by the FSA ops data monitoring system, and a notification is sent to APHA in the form of a trigger report. The changing pattern of trigger reports from year to year provides a complex picture, which is likely to reflect factors including;

- a) There will be 'natural' fluctuations in the performance of the broiler flocks, and the this will be reflected in the response of the population of animals (the sample) over time resulting from production system factors (nutrition, vaccination, genotype selection) which will influence the 'response of the population' to management factors.
- b) There have been changes in the structure of the poultry industry in the period 2010 2014, with mergers, increased production in some companies, and altered priorities in, for example, the weight of birds supplied and the proportion of the flocks thinned. This will have affected the management trends within the industry but is not unexpected, in an industry which is responsive to consumer demands and production pressure.
- c) The disease background in the flock which has not been constant across the period (2010 to 2014) and so the trigger report levels reflect closely the incidence of disease, and the effectiveness of response to disease challenge.

#### What happens when a 'trigger is raised'?

Where levels of the trigger level conditions exceed a certain threshold, the keeper of the animals is alerted. Where poor welfare is suspected, the Official Veterinarian can advise the keeper of the animals and enforcement action by the local veterinary agency Animal and Plant Health Agency (APHA), Food Standards Agency Operation (FSA Ops) and by Local Authorities can result. APHA and FSA have inspection regimes and data handling systems to communicate information relating to poor welfare between the slaughterhouse and the producer.

Action may include a visit to the production site by APHA veterinary officers, and the creation of a written action plan in conjunction with the keeper, and APHA may, in addition, carry out a number of random welfare inspections. Each welfare inspection records scores against a number of welfare criteria, and an overall score based on the 'lowest scoring' of these. This overall score identifies:

- (A) Full compliance with legislation and the welfare codes of practice
- (B) Compliance with legislation only
- (C) Non-compliance with legislation
- (D) Non-compliance with legislation and presence of unnecessary pain or distress

# How variable are levels of individual trigger conditions from year to year, and throughout each year?

There is variability in the number of trigger reports generated from year to year and from quarter to quarter. To illustrate this, choosing to use Ascites, Dermatitis and Respiratory Disease (measures which commonly generate trigger reports) **Figure 1** indicates the variability in Ascites, Dermatitis and Respiratory Disease (ratio of trigger reports:batches sampled) for 10 production companies with a range of production output across the period 2010 to 2014.



## What are the main resolutions or actions by the producers resulting from the use of this welfare monitoring data?

If trigger thresholds are exceeded, the government agency issues a trigger report to the producer, and the producer creates an action plan - a written description of steps to be taken to address the condition raised, which is submitted to the veterinary officer investigating the trigger report. Ranking of key action plan elements (phrases which commonly occur in action plans) has enabled the common action plan features to be identified.

Figure 2 shows the frequency of use of key words/phrases identified in action plans used expressed as 'wordles' to enable the relative importance of key action plan words to be seen.





A Common conditions



B Common 'responses' (actions)

**Figure 2** Common conditions (A - upper text), and common 'action plan' words and phrases (B - lower text) for the period 2010 to 2014 ranked by decreasing occurrence in graphical form, and expressed as 'wordles' which gives prominence to the terms in proportion to their occurrence rate.

#### Response to the trigger system to date

To date the meat chicken welfare trigger system indicates itself to be successful in many respects. There have been systematic changes in the rate of some conditions, and this has been detected through use of the trigger system, and this now enables potential for targeted action to explore, and alter, the underlying causes of these changes. Analysis of response to triggers by following a number of farms has shown that that flocks show improvement after a trigger has been raised.

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## O<sup>42</sup> Effects of Slatted Floor Housing on Animal Welfare in Broiler Production

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#### Abstract

Deep litter floor housing system is common for raising broiler production. Although cage and slatted floor housing for broiler production have been available for many years, they were not widely adopted because heavy broiler chickens are prone to leg deformities and breast blisters. These problems have adversely affected broiler meat quality. Currently, cage housing system is becoming more popular in broiler production. But limited space area and inappropriate condition for their natural behaviour in cage system has been criticized for the animal welfare like in egg production. In that reason it can be said that slatted floor housing can be more suitable for alternative floor in broiler production. This study was made to investigate the effects of slatted floor housing on broiler welfare. Animal based welfare parameters were measured to investigate welfare level in the group of deep litter (control) and slatted floor (experiment).

Key Words: Broiler, floor, housing, animal welfare.

## O<sup>43</sup> Effect of Maternal Stress on Relative Asymmetry and Fear Behaviour of Broiles Reared Under Harsh Environmental Conditions

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#### Abstract

Corticosterone (CORT) can be transferred from the mother to the egg and deposited into egg. Increasing CORT concentration in the eggs via stress experienced by mother during breeding, may affect offspring' development. This study aimed to evaluate effects of maternal stress (MCORT) which created by CORT supplementation in the diet of broiler breeder hens on relative asymmetry and fear behaviour of broilers reared at the higher stocking density. For this purpose, a total of 200 Ross broiler breeder hens at 42 wk of age were randomly divided into two groups as control (CONT) and MCORT which fed 2 mg CORT/hen/d for 14 d. Eggs were collected and incubated. Chicks obtained from both groups were reared at optimum (SD<sub>o</sub>) or higher stocking densities (SD<sub>H</sub>). Relative asymmetry of morphological traits and tonic immobility (TI) duration as an indicator of fear behavior, were determined.

Relative asymmetry of shank and face lenghts and mean relative asymmetry of MCORT broilers were higher than CONT. Stocking density and age did not affect relative asymmetry of shank, middle toe and face lenghts and mean relative asymmetry.

TI duration increased in CONT broilers compared to MCORT. There was a significant interaction between maternal effect and stocking density on TI duration which indicated that MCORT broilers had shorter durations of TI regardless of stocking density.

We concluded that the higher relative asymmetry and lower tonic immobility duration obtained for MCORT broilers indicated long-term effect of maternal stress.

Keywords: maternal stress, corticosterone, stocking density, broiler



## O<sup>44</sup> Poultry Investment Report for East Part(*Malatya, Elazığ, Bingöl, Tunceli*) of Turkey

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#### Abstract

In this study, "Poultry Industry" were examined in TRB1 Region (Malatya, Elazığ, Bingöl, Tunceli). Poultry group represents to grow chicken, turkey, goose, duck, ostrich, partridge, pheasant and quail enters various bird species for hobby or commercial purposes. Since the economic value of chickens are higher, raised and widely consumed for their meat and eggs. Ducks and other poultry breeding geese done as a hobby, it is excluded in this study.

In fact, eggs and broiler production is observed, to be considered as a sector in its own in the world and in our country. This production also contributes to the development of construction materials and tools, such as feed, cage, waterers and feeder, vaccines and also of the pharmaceutical industry.

Poultry Industry is developing since the productivity can be satisfied with less effort and maintainanace. That sector is raising in rural areas and having an important role for providing the need of protein for the country with their eggs and meat. Red meat and white meat can be substituted, especially more of the population is in low-income families, and they meet the protein requirements with white meat.

In this study, the poultry sector TRB1 has been examined by compiling surveys and scanning thesis work carried out in this region. The quantitative data of the sector has been compiled, the trend of the industry shown, and a feasibility analysis has given for helping investor.

## O<sup>45</sup> Further Understanding IBV is Essential to Effectively Control Respiratory Disease in Chickens

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Eight decades after its first report in 1931 (12), infectious bronchitis (IB) continues to severely affect chicken meat production, the most important protein source for human consumption worldwide. Some of the enormous economic losses in broiler chickens due to IB are caused by reduction in weight gain and feed conversion efficiency, increased condemnations at processing plants and increased deaths at arrival at the slaughter plant. In layer and breeder hens production is affected by pronounced declines in egg production and quality. For example, data from a large broiler integrator in Brazil indicated that at a production level of 1.65 billion broilers/year, the total financial losses due to IB for this company were estimated at \$12,705,514/year (1,2). A deep understanding of the biology of IBV by poultry professionals is without doubt essential for effective control of the disease.

IB is caused by infectious bronchitis virus (IBV) a member of the Coronavirus genus in the family Coronaviridae. The avian coronaviruses, which include IBV, belong to the gammacoronavirus group (3,4,9). The phenotypic characteristics and genome organization of coronaviruses, including IBV, have been comprehensively reviewed by others (3,4,9,13). The strategy used by IBV for successful adaptation in the host environment was first revealed in 1956, when Jungherr et al. (8) demonstrated antigenic differences between virus isolates obtained in the states of Massachusetts and Connecticut. Since then, numerous conventional and molecular epidemiological studies have confirmed the ability of IBV to rapidly evolve and successfully circumvent extensive vaccination programs which include a multiplicity of serotype-specific vaccines. For example, numerous IBV outbreaks have occurred in Turkey despite extensive vaccination. IBV isolates from these disease outbreaks have been attributed to novel IBV variants similar to IS/1494/06 -type (5).

Even though attenuated IBV vaccines provide effective protection against homologous challenge, accumulating evidence indicates that these vaccines may also be contributing to the emergence and circulation of vaccine-like viruses in the poultry industry (14,15).Viral populations differing from the predominant vaccine population of IBV Arkansas (Ark) serotype attenuated vaccine strains have been shown to emerge during a single passage in chickens (10,16), providing a striking model to understand the mechanisms driving IBV evolution. Based on spike protein S1 gene sequences, we previously identified five distinct virus subpopulations in ArkDPI-derivedvaccines that became rapidly positively selected in the chicken upper respiratory tract, whereas the predominant IBV phenotype contained in the embryo-attenuated vaccines was negatively selected (7,16). Differences in frequencies of phenotypes within IBV populations are associated with differences in the behavior of these viruses in the host (11). From an applied perspective, genetic and phenotypic shifts occurring in Ark-type IBV vaccine populations during replication in chickens are likely responsible for the emergence of Ark-like viruses in the U.S. poultry industry.


The establishment of distinct IBV subpopulations has been shown to be influenced by differing immune selective pressure during host invasion. Several host and environmental factors influence selective pressure and include for example strength and specificity of vaccine-induced immune responses. Indeed, chickens vaccinated with different commercially available IBV attenuated vaccines, even vaccines originating from the same original virus seed, display significant differences in the induction of immune responses. For example, we have found differing specific B lymphocyte responses in the Harderian gland in chickens vaccinated with different ArkDPI-derived vaccines, i.e., the challenge virus would encounter differing immune selective pressure during invasion. Chickens with the strongest immune response (vaccine A) were able to successfully impede replication of the challenge virus in most chickens and, based on S1 sequences, only the virus subpopulation predominant in the challenge strain was detected in a few IBV positive birds. In contrast, in chickens showing less than optimal specific immune responses (vaccines B and C) IBV was detected in most chickens and populations different from the predominant one in the challenge strain were selected and became predominant. These results provided scientific evidence for the assumption that poor vaccination contributes to the emergence of new IBV strains (15).

In another study, we followed changes in a portion of the S1 gene sequence of the dominant populations of an IBV Ark vaccine strain during serial passages in immunocompetent chickens and in chickens infected with viruses causing immunodeficiency [chicken anaemia virus (CAV) and infectious bursal disease virus (IBDV)]. The IBV-Ark vaccine was applied ocularly and tears were collected from infected chickens for subsequent ocular inoculation in passages to follow. The dominant S1 genotype of the vaccine strain was rapidly negatively selected in all chicken groups. During the first passage both in immunocompetent and immunodeficient chickens the same IBV subpopulations previously reported (7) emerged followed by establishment of one or two predominant populations after further passages. Only when the subpopulation previously designated C2 became established in immunodeficient chickens IBV was maintained through more than four passages. These results indicated that selection does not cease in immunodeficient chickens and that phenotype C2 may show a distinct adaptation to this environment. Other subpopulations initially became established in immunocompetent birds but became extinct after only a few succeeding passages. These findings constituted further evidence for phenotypic drift occurring mainly as a result of selection (6).

The challenges posed by IBV's plasticity establish an urgent need for novel approaches to effectively control the disease and associated economic losses. A more recently developed control strategy involves vaccination with one serotype followed by vaccination with anotherIBV serotype. Supporters of this approach indicate that chickens not only develop immunity to the serotypes in both vaccines but also cross reacting antibodies to other IB serotypesIrrespective of the possible protective benefits of using combinations of different attenuated IBV strains to protect chickens against IBV, the main disadvantages of attenuated vaccines persistincluding (1) risk of recombination between live attenuated IBV vaccines and/or wild IBV strains; (2) introduction of exotic live IBV strains; and (3) risk of emergence of vaccine-likestrains resulting from point mutations. Other vaccine options, including recombinant vaccinesexpressing broadly protective immunogens, seem more promising.

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## O<sup>46</sup> Intestinal Microbiome of Broilers and It's Interaction with Health and Disease Status of the Host

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#### Abstract

In Turkey, *Salmonella* infections are the most observed infections of poultry industry and also it is the most important disease fort the public health and economical loss. Antibiotic usage is the most important treatment method for struggling the disease. Nevertheless the bacterial resistance to antibiotics leads to alternative treatment regimens. Considering the relations between microorganisms recently the most attractive word microbiome seems to gets importance. The technological improvements in biological areas leads to performing laborious metagenomic analysis in very short time with a little cost and high accuracy. With this extent the new sequencing systems help us to correlate microbial spectrum between animals and humans and also between the diseases. Also with these systems there is no need to make any amplifications and conventional culture techniques for determining the microorganismal taxon. This feature gives us the opportunity for determining the microbiome of animal, human, food and environment. With the known microbiota the colonization of bacteria such as *Salmonella* and *Clostridium perfringens* in poultry could be inhibited and the need to antibiotic usage would be decreased.

## O<sup>47</sup> Rapid Detection of Infectious Bronchitis Virus (AvCoV-IBV) by TaqMan Real Time RT-PCR and Phylogenetic Analysis of S1 Gene Variation

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#### Abstract

Factors like intensive breeding and vaccinations may cause decrease in resistance facilitating the entrance and trigerring of infectious agents. Amongst these agents, infectious bronchitis virus (AvCoV-IBV) is an RNA virus and therefore is prone to mutation and development of variant strains.

The aim of this study (TUBİTAK, 113O411; COST action FA 1207) was rapid detection, isolation and investigation of S1 gene variants of infectious bronchitis virus (AvCoV-IBV) in broilers and layer chickens. For this, tracheal swabs from chickes of the 14 layer farms and tracheal tissue samples from broilers with respiratory problems from 38 broiler farms located in Marmara, western Black Sea, Mediterranean and Inner Anatolia region. Rapid detection of IBV was assessed by a real-time RT-PCR-Prob assay (Callison et al., 2006). Samples giving low CT value by real-time PCR were inoculated into 9-11 days embriyonated SPF chciken eggs. Samples with low CT value was further subjected to sequencing PCR by using the primers targeted to S1 gene of IBV (Dr. I. Monne, personnel communication through COST action FA 1207 and Worthington et al., 2008). Phylogenetic tree was generated by alignment of the sequences with known strains of IBV submitted to GeneBank by other investigators from Turkey and other countries.

IBV was detected in 9 layer farms and 29 broiler farms by TaqMan Real-Time RT-PCR. According to the partial S1 gene sequences and phylogenetic analysis of IBVs detected in chickens originated from 7 layer farms and 18 broiler farms; the partial S1 gene of IBVs detected in 3 layer farms were similar to IS/236 isolate (99,7%), Ma5 strain (99,2- 99,7%), China-W93 isolate (99-99,5%), H120 strain (99-99,5%), NGA/310/2006 strain (99,5-100%), Mass41 strain (97,5-98,1%), Connecticut vaccine strain (96,4-96,9%) and Florida 18288 strain (95,9-96,4%). In addition, the partial S1 gene of IBVs detected in 15 broiler farms were similar (94,9-99,7%) to IBVs previously reported from Turkey and Israel variant-2 strains (KF007927, EU780077, JX027070, JX173488). However, the partial S1 gene of IBVs detected in 7 layer farms were different than what was found in broilers but similar (95.1-% 99.1%) to strains previously reported from Iran, India and China, and also gen cluster of Israil Variant-1 and 4/91.

In cocnlusion, different IBV strains (including vaccine strains) are circulating in chicken farms in Turkey and may pose risk of mutation and recombination amongst these strains.



## O<sup>48</sup> Effects of Heat Stress on Production Performance, Intestinal Integrity and Meat Quality in Broilers And Potential Nutritional Interventions

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#### Summary

World-wide production of poultry meat is rapidly growing, mainly in (sub)-tropical areas. Modern broiler breeds have a continuously increasing potential for fast growth and high feed efficiency, which coincides with a higher sensitivity to heat stress. Housing these broilers at high ambient temperatures not only impairs production performance, but also adversely affects intestinal integrity, immune response, and meat quality. Several dietary interventions are presented to support the birds' resilience against heat stress, including the effect of several feed additives. Feed additives that alleviate the consequences of heat stress, among which phytogenic feed additives, generally exert clear anti-oxidant effects.

#### Introduction

Recently the OECD/FAO (2014) indicated that in 2020 poultry meat will have become the largest meat sector. During the next decade, poultry meat is expected to capture half of the increase in global meat production. As this expansion will mainly be concentrated in (sub)-tropical countries, the risk of heat stress to broiler meat production increases. Adverse effects of heat stress in poultry will be exacerbated by the increasing growth potential of modern broiler breeds(Yearly it takes 0.74 days less to reach 2.27 kg body weight in broilers, whereas feed conversion ratios are improving by 0.025 (McKay as cited by Gous, 2010).. The continuously improving genetic potential of broilers might have a negative impact on the bird's resilience, i.e. their possibilities to cope with different challenges. Van der Klis (2014) summarized indications for such adverse effects:

- 1) Selection for enhanced performance traits reduced adaptive immunity (Quereshi and Havenstein, 1994);
- 2) Mortality and incidence of metabolic diseases has increased in parallel with growth rate (Kalmar et al., 2013);
- 3) Modern fast growing broilers are more susceptible to heat stress (Gous, 2010);
- 4) Heavy weight and/or rapid growth rate have been suggested as causal factors for two recently described myopathies (Sihvo et al., 2013).

To reduce adverse effects of heat stress and design effective intervention strategies, it is important to understand the basic mechanisms of heat stress. Some data indicate that heat stress in broilers occurs at much lower temperatures than generally perceived. A 3-year survey in the United Kingdom on mortality of broilers during transport from the farm to the slaughter house showed that the incidence of dead on arrivals sharply increased already when the outside temperature exceeded 20C (Warriss et al. 2005). Although their observations on the effect of ambient temperature during transport were concurrent with poor ventilation conditions in transport containers, it is a clear indication for the stress sensitivity of modern broilers.

#### **Consequences of heat stress**

Lara and Rostagno (2013) summarized the effects of chronic heat stress on broiler performance, being:

- reduced feed intake and body weight gain;
- increased feed conversion ratio;
- decreased breast muscle and increased thigh muscle proportions;
- reduced protein;
- reduced meat quality.

Reduced feed intake of broilers during heat stress only accounts for 25% to 35% of the lower body weight gain as was shown in studies with pair-fed broilers. Feed intake effects therefore seem to be overestimated. The major reduction in growth performance is related to stress as such (Bonnet et al., 1997; Daghir, 2009). During chronic heat stress plasma cortisol is increased and thyroid hormone levels are reduced (e.g. Sohail et al., 2010). These elevated plasma cortisol levels stimulate muscle catabolism and lipid peroxidation in muscle tissues, which was concluded from increased malondialdehyde (MDA) contents in breast muscle of broilers (Zhang et al. 2011). Azad et al. (2009) showed that lipid peroxidation in pectoralis muscle of broilers increases with the severity of heat stress during the last two weeks pre-slaughter. Moreover, they showed that rectal temperature of heat stressed broilers was increased by approx. 2C comparing broilers housed at thermoneutral temperature and broilers housed at constant 34C. Niu et al. (2009) and Song et al. (2014) showed that heat stress additionally impairs immune response and intestinal integrity. The latter effect was related to lipid peroxidation in the enterocytes. Gu et al. (2012) indicated that heat shock proteins (HSP70, a group of highly conserved protective proteins, involved in cell protection and cell repair) play an essential role alleviating heat stress response, as they stimulate antioxidant enzyme activities, relieving oxidative damage in intestinal mucosal cells during heat stress. Adverse effects of heat stress on intestinal integrity may account for reported higher translocation of Salmonella enteritidis, resulting in intestinal inflammation and increased Salmonella counts in tissues after heat stress (Quanteiro-Filho et al. 2012). Additionally, Bonnett et al. (1997) showed that nutrient digestibilities were reduced, which supports the need of using feed ingredients with a higher digestibility (and therefore dietary nutrient concentration will require the use of high quality feedstuffs) or feed additives that support nutrient digestion.

Additionally, it is demonstrated in many papers that heat stress exerts direct adverse effects on meat quality. For instance Dai et al. (2009) showed that housing broilers at 28C from 35 to 42 days of age reduced breast meat pH and water holding capacity and increased breast meat discoloration, compared to broilers housed at 23C and Zhang et al. (2012) showed that both constant (34C) and cyclic high temperatures (36C during 6h and 23C during rest of the day) from 29 to 42 days adversely affected these meat quality parameters, whereas effects of constant high temperature was much more detrimental than during cyclic high temperatures. Therefore, heat stress has a negative impact on consumer perception of the final product.

#### Nutritional strategies to reduce heat stress

In 2010, Gous summarized main nutritional strategies to alleviate heat load of broilers:

- 1) the use of high density feeds;
- 2) increased protein to energy ratio;
- 3) improving amino acid profile;
- 4) changing the energy source from carbohydrates to fat;



- 5) feed form and restricted feed allowance;
- 6) addition of vitamins;
- 7) modifying the cation:anion balance.

Although effects of nutrient concentration on heat load of broilers are limited, dietary concentration reduces energy expenditure for nutrient intake and its positive effects are therefore similar to feeding good quality pellets. Although it is clear that limiting excess protein and optimizing amino acid profile minimize metabolic energy costs to excrete surplus nitrogen, the effect of heat stress on the optimum amino acid profile is not yet known.

Gous (2010) indicated that although a higher fat content at the expense of carbohydrates will reduce metabolic heat production, effects are limited when relying on normal feed ingredients. It is well-accepted that management factors like feed withdrawal 4 to 6 h prior to the hottest period of the day limit heat increment of feeding. However, broilers will only benefit from temporary feed withdrawal if the ambient temperature during night-time is substantially lower than during the day (cyclic heat stress) to enable compensatory nutrient intake during the cooler periods of the day. Heat-stressed birds dissipate up to 80% of their heat production by evaporative cooling by panting (Van Kampen, as cited by Gous, 2010). As panting increases  $CO_2$  losses, heat-stressed birds will benefit from a highr cation: anion balance.

Apart from optimizing feed composition and structure, several (classes of) feed additives have been mentioned in scientific literature to alleviate (the consequences of) heat stress. Papers indicate that the efficacy of such additives is focused on their anti-oxidant effects. Heat-stress induces oxidative processes in the enterocytes as discussed in 'consequences of heat stress'. Therefore, increased levels of dietary antioxidants, like a combination of vitamins A and E, reduce lipid peroxidation during heat stress (Sahin et al., 2002) as well as plants and plant extracts like ginger root powder and its essential oils (Habibi et al., 2014). Moreover, adding vitamin E improves immune response of heat stressed broilers (Niu et al., 2009). Placha et al. (2014) showed that *thyme oil* improved intestinal antioxidant status, reduced MDA content in the enterocytes and improved intestinal integrity. Data show synergistic effects between several antioxidative feed ingredients. Glutamine is considered to be a conditionally essential amino acid and has been shown to improve heat-stress resilience of broilers. Dietary glutamine improved growth performance and meat quality of broilers subjected to heat stress in a dose dependent manner (Dai et al., 2009). In addition Gu et al. (2012) showed that glutamine enhanced the expression of HSP70 in jejunal mucosa after acute heat stress, protecting it from heat stress injury via increased levels of anti-oxidant enzymes in the jejunal tissue. Finally, Yesilbag et al. (2011) showed that an increased anti-oxidant status in meat by feeding broilers diets supplemented with rosemary or its essential oils improved meat quality and shelf life.

Feed additives that improve resilience against heat stress, among which phytogenic feed additives, generally exert clear anti-oxidant effects. Therefore, antioxidant effects seem to be the most important effects to focus on when developing feed additives to improve heat stress resilience.

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## O<sup>49</sup> Effect of *in ovo* and Post Hatch Synbiotic Administration on Broiler Performance, Intestinal Histomorphology and Microflora from 0 To 21 Days of Age

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#### Abstract

This study was conducted to investigate the effect of intra-amniotic synbiotic inclusion and continued receiving it in the diet on broiler performance, intestinal epithelium integrity and cecum microflora of broiler chickens during 21 d experimental period. In Experiment 1, a total of 510 eggs containing viable embryos were divided into 3 groups of 170 eggs each. The first group was not injected and served as a negative control (NC). Second group was injected with 0.9% NaCI and served as positive control (PC). The last group was injected with 0.5% inulin and 1 x 10<sup>6</sup> Enterococcus faecium NCIMB 10415 containing solutions and served as synbiotic injected group (S). Hatchlings of non-injected and synbiotic injected groups were selected for Experiment 2. Each group was divided into 2 new groups and birds were offered basal diet and synbiotic supplemented diet as follows: 1) NC-Basal: non-injected birds fed basal diet; 2) NC-Syn: noninjected birds fed 1% inulin and  $2 \times 10^9 E$ . faecium cfu/kg feed; 3) S-Basal: synbiotic injected birds fed basal diet; 4) S-Syn: synbiotic injected birds fed 1% inulin and  $2 \times 10^9 E$ . faecium cfu/ kg feed. Inulin and E. faecium incorporation to the eggs on E17, did not affect hatchability and hatching weight of the birds. In addition, intra-amniotic synbiotic administration nor continued dietary synbiotic treatments did not affect body weight gain, feed intake and feed conversion ratio during 21 d experimental period. In ovo synbiotic administration and following dietary synbiotic treatment caused an increase in ileum villus height versus to NC-Basal group birds on d 21. In addition, intra-amniotic synbiotic inoculation and continued dietary synbiotic treatment significantly increased Lactobacillus count and decreased coliform population in cecum on d 21. Our results indicate that intra-amniotic administration and dietary synbiotic improved broiler intestinal integrity and increased cecal beneficial microflora population.

Key words: broiler, in ovo feeding, synbiotic, intestinal histomorphology, cecum microflora

#### Introduction

In modern broiler production, newly hatched birds contact with the bacteria in hatchery and house environment instead of directly hen or nest material (1). Therefore early colonization of the hatchling intestine by beneficial bacteria is required to maintain gut microflora balance, reducing the likelihood of enteric invasion of pathogens by competitive exclusion and developing disease resistance (2). In ovo feeding technique was developed to improve intestinal functionality of



intestine and overcome the physiological limitations due to the delayed access to feed after posthatch period (3). According to this method, nutrient solutions are directly inserted to amniotic fluid to deliver essential nutrient to intestine before hatch. Previous studies have shown that many nutrient, such as carbohydrates (4,5,6), vitamin and minerals (7), can be incorporated to amnion during the last quarter of the incubation. In addition, recent studies have demonstrated that, probiotics, prebiotics and synbiotics could be administered to chick eggs that contain viable embryo. *Enterococcus faecium*, a lactic acid bacterium and normal inhabitant in the gut, is a probiotic that may be useful in animal health (8,9). Intra-amniotic *E. faecium* inoculation to the eggs and its presence and viability in chicks' ceca was clear demonstrated by De Oliveira et al. (1). In addition they revealed that *Salmonella Enteritidis* was decreased by the in ovo *E. faecium* administration and continued receiving it in the diet (1).

Inulin is a mixture of linear polymers and oligomers of fructose linked by a  $\beta(2-1)$  glycosidic linkage (10). Because of the configuration, inulin is not hydrolysed by digestive enzymes and positively affect the host by selectively stimulating the growth and activity of one or limited number of bacteria in the intestine (10). Previous studies have revealed that the dietary prebiotics may increase the intestinal beneficial bacteria population (11), alter the cecal microbial activity (12), and improve the gut integrity (13), and digestibility of proteins and fats in a maizesoybean meal based diet of broiler chickens (14). In addition, it has been showed that in ovo inulin (15) and mannan oligosaccharide (16) inclusion has beneficial effect on gastrointestinal functionality and development. The importance of early established beneficial microflora in the chicken intestine and its beneficial effect on intestinal health has long been known. Based on the suggested favorable effects of intra-amniotic probiotic or prebiotic administrations, we hypothesized that in ovo synbiotic inclusion and continued receiving it in the diet may be an effective way to maintain the intestinal epithelium integrity and cecum microflora of broiler chickens from 0 to 21 days of age.

#### **Material and Methods**

#### **Experiment 1**

#### **Incubation Procedures and In Ovo Administration**

A total of 900 eggs (Ross 308) were obtained from a 33 wk old maternal flock in a commercial hatchery (Beypilic A.Ş., Bolu, Turkey). At arrival, all the eggs were individually weighted and 573 eggs with an average weight of  $59.48 \pm 2.54$  g (Mean  $\pm$  SD) were selected to place the incubator. The eggs were divided into 6 flats of 12 trays of incubator. Each tray contains 8 individual rows which holds 6 eggs with similar average egg weights per row. The eggs were incubated under standard conditions. Prior to the injection, at  $17^{th}$  d of incubation (E17), the eggs were candled and those unfertilized or with dead embryos were discarded and total of 510 eggs containing viable embryos were divided into 3 groups of 170 eggs each. At the day of injection eggs were removed from the incubator, candled and marked on the side of the egg to locate the amnion. The eggs were disinfected and then a hole was punched into the shell at the side of aircell chamber with a sterile 21-gauge needle. The first group was not injected and served as a negative control (NC). Second group was injected with 0.9% NaCI and served as positive control (PC). The last group was injected with 0.5% inulin (Orafti IPS, Beneo, Oreye, Belgium) and 1 x 10<sup>6</sup> Enterococcus faecium NCIMB 10415 (Cylactin ME20, DSM Nutritional Products, Basel, Switzerland) containing solutions (Table 1). The eggs were injected with specific solutions, at the volume of 0.6 mL, with self-refilling syringes (Socorex, Ecublens, Switzerland). All eggs remained outside the incubator for the same amount of time, including the NC treatment group. After injection, the injection hole was sealed and the eggs were placed in separated hatching baskets as in the each flat rows.

#### **Hatch Sampling**

At hatch, the number of live hatched chicks and nonhatched chicks was counted to determine hatchability of fertile eggs (%). Nonhatched eggs were opened to determine cause of death. After, all hatched chicks were weighed and 12 chicks from each treatment were randomly selected to determine the internal organ and yolk weights.

#### **Experiment 2**

#### **Birds and Management**

Hatchlings of non-injected and synbiotic injected groups were selected for Experiment 2. Each group was divided into 2 new groups and birds were offered basal diet and synbiotic supplemented diet, as follows NC-Basal: non-injected birds fed basal diet; NC-Syn: non-injected birds fed 1% inulin and  $2 \times 10^9$  Enterococcus faecium cfu/kg feed; S-Basal: synbiotic injected birds fed basal diet; S-Syn: synbiotic injected birds fed 1% inulin and  $2 \times 10^9$  Enterococcus faecium cfu/kg feed (Table 1). A total of 196 broiler chicken (Ross 308) were randomly allocated to 4 experimental groups with 7 replicate pens (90  $\times$  80 cm) containing 7 birds each. The temperature for the first week was ~32-35°C and then gradually reduced to 20°C until the end of the experiment. The relative humidity of the house during the experiment was  $50 \pm 5\%$ . The house was artificially ventilated by ventilators and during the study continuous light regimens were provided. Chicks were vaccinated at hatch against the Newcastle disease virus (La Sota strain) and the infectious bronchitis virus. All experimental procedures were approved by The Animal Ethics Committee of the Ankara University. The starter and grower diets were based on the maize-soybean meal and were offered to birds from 0-14 and 15-21 days of age, respectively (Table 2). All diets were formulated to meet or exceed NRC (1994) nutrient recommendations. Each pen was equipped with a manual plastic feeder and an automatic nipple drinker. Water and diets (in mash form) were provided ad libitum throughout the experimental period.

#### Sampling Procedures

At d 21, one bird from each replicate was selected according to the average BW of each treatment group. Birds were slaughtered by exsanguination and the intestinal tract was removed immediately. The tissue samples for histomorphological analysis were taken from the jejunum and ileum. Approximately 1 cm length of the mucosal segments of jejunum and ileum was excised and tissue samples were flushed with saline solution to remove adherent intestinal contents and fixed in 10% neutral buffered formaline solution for 24h. In addition, at d 21, one gram of cecum content from each selected bird was collected in sterile tubes for bacterial enumeration.

#### **Histomorphologic Measurements**

Tissue samples in the formaline solution were dehydrated in graded ethanol solutions, cleared with xylol and then were embedded in paraffin. The intestinal segments were sectioned at a thickness of 5  $\mu$ m with microtome. Cross sections were prepared and stained with combined alcian blue-periodic acid Schiff (AB-PAS) reagents in order to determine the jejunal and ileal morphometry (17). Villus height was measured from the top of the villus to crypt mouth and crypt



depth was defined as distance between basements of the crypt-to-crypt mouth. A total of 10 welloriented villus and crypt were selected randomly for histological measurements. Histological sections were examined under the light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with Leica DFC450 (Leica Microsystems, Heerbrug, Germany) digital microscope camera. The images were evaluated using ImageJ software (Image J, US National Institutes of Health, Bethesda, MD, USA).

#### **Determination of Bacterial Population in Cecum**

One gram of fresh cecal digesta was transferred to 9 mL of sterile physiological saline solution and homogenized. Inoculants were serially diluted up to  $10^{-8}$ . Subsequently, dilutions of  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$  were inoculated (100 µL of each dilution) to appropriate selective agar media to determine coliform and *Lactobacillus* counts. Mac Conkey agar (Merck Millipore) and MRS agar (Merck Millipore) were used to enumerate coliform and *Lactobacillus*, respectively. All dilutions were inoculated to selective agars in triplicate. Bacterial colonies were counted and averaged. Data were expressed as  $\log_{10}$  colony-forming units (cfu) per gram of cecal digesta.

#### Statistical analysis

Data were analysed using the ANOVA procedure of the SPSS version 14.01 (SPSS Inc., Chicago, IL, USA). Significant differences among treatment groups were tested by Tukey's multiple range tests. Statistical differences were considered significant at P < 0.05.

#### **Results and Discussion**

The effects of intra-amniotic synbiotic administration on hatchability (%), hatching weight and internal organ and yolk weights are shown in Table 3. Inulin and E. faecium incorporation to the eggs on E17, did not affect hatchability and hatching weight of the birds. In agreement with these findings, previous studies have reported that  $5 \times 10^9$  cfu/per egg *E. faecium* (1), 4% inulin (15) and 0.1% mannan oligosaccharide (16) did not reduce hatchability and hatching weight of the chickens in comparison with negative control. However, combined intra-amniotic application of inulin and E. faecium have not heretofore been studied or reported. Our results indicated that combined use of inulin and E. faecium might be used in practice without any negative effect on hatchability and hatchling performance. Intra-amniotic synbiotic administration nor continued dietary synbiotic treatments did not affect body weight gain, feed intake and feed conversion ratio during 21 d experimental period (Table 4). Similarly, Maiorano et al. (18) suggested that, in ovo prebiotic and synbiotic administration and post-hatch basal diet consumption did not affect the broiler performance parameters on d 42. However limited data exist regarding the effects of in ovo probiotic, prebiotic or synbiotic administration and post-hatch continued synbiotic consumption on broiler performance. Contrary to our results, several studies suggested that (19,20), dietary synbiotic supplementation increased the broiler performance in comparison to the control birds. Patterson and Burkholder (21) suggested that environmental and stress status influence efficacy of prebiotics and probiotics, and are more effective when the animal is producing well below its genetic potential. In addition, it is necessary to mention that the current study was conducted under good hygienic conditions and eggs were obtained from young breeders flock. As a result, the need for dietary feed additives to increase the production performance might be decreased to minimum. The morphological changes in the small intestine, such as increasing villus height and VH:CD ratio might have beneficial effects on birds performance. So that, these changes enhance the absorptive surface area that is prominent when the alternative growth stimulators are applied. Morphological measurements of the jejunum and ileum are shown

in Table 5. In current study, in ovo synbiotic administration and following dietary synbiotic treatment caused an increase in ileum villus height versus to NC-Basal group birds. Similarly to our study results, Awad et al. (19) revealed that ileal villus height was significantly higher for the birds fed synbiotic supplemented diets on d 35 of the experiment. Our results showed that dietary synbiotic supplementation (NC-Syn and S-Syn) decreased cecal coliform count on d 21. In addition, intra-amniotic synbiotic inoculation and continued dietary synbiotic treatment significantly increased *Lactobacillus* count in cecum on d 21 (Table 6). More recently, Mookiah et al. (20), reported a significant increase in *Lactobacillus* count and decrease in *E.coli* count of the broiler cecum on d 21 and d 42. It is widely accepted that the intestinal microflora and its metabolic activity have significant effect on the broiler health and the performance.

The present study demonstrated that, intra-amniotic *E. faecium* and inulin inoculation and continued receiving in the diets improved the broiler gut health with accompanying increase in ileum villus height and cecum *Lactobacillus* count and decrease in coliform count at the end of the 21 d experimental period.

#### Acknowledgments

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Treatment	Description	In ovo 0.9% NaCI inoculated	In ovo Synbiotic inoculated	
Experiment 1				
(Expl)				
NC	Negative Control	-	-	
PC	Positive Control	+	-	
S	Synbiotic Group	-	+	
Treatment	Description	In ovo Synbiotic inoculated birds	Basal Diet	Synbiotic Supplemented Diets
Experiment 2 (Exp2)				
NC-Basal	NC birds of Exp1 – fed basal diet	-	+	-
S-Basal	In ovo synbiotic inoculated birds-fed basal	+	+	-
NC-Syn	NC birds of Exp1 - fed synbiotic supplemented diets	-	-	+
S-Syn	In ovo synbiotic inoculated birds - fed synbiotic supplemented	+	-	+

#### **Table 1.** List of treatments of Experiment 1 and Experiment 2.



	Starter		Grower		
	0 to 1	4 d	15 to 21 d		
Item	Basal Diet	Synbiotic	Basal Diet	Synbiotic	
Ingredient, %					
Corn	50.03	48.83	55.10	53.80	
Soybean meal	30.00	30.00	24.00	24.00	
Soybean (Full fat)	13.00	13.00	13.00	13.00	
Vegetable oil	2.50	2.70	3.90	4.20	
Limestone	0.86	0.85	0.80	0.80	
Dicalcium phosphate	2.25	2.25	2.00	2.00	
DL-Methionine (98%)	0.40	0.40	0.31	0.31	
L-Lysine-HCl (78%)	0.26	0.26	0.19	0.18	
L-Threonine	0.15	0.15	0.15	0.15	
Salt	0.35	0.35	0.35	0.35	
Vitamin premix <sup>2</sup>	0.10	0.10	0.10	0.10	
Mineral premix <sup>3</sup>	0.10	0.10	0.10	0.10	
Inulin <sup>4</sup>	0.00	1.00	0.00	1.00	
Enterococcus faecium <sup>5</sup>	0.00	0.01	0.00	0.01	
Total	100.00	100.00	100.00	100.00	
Chemical composition (calculated)					
ME, kcal/kg	3,037	3,015	3,196	3,180	
CP, %	22.70	22.60	20.30	20.20	
Lysine, %	1.43	1.43	1.23	1.23	
Methionine + cysteine, %	1.10	1.09	0.95	0.95	
Calcium, %	1.00	0.99	0.90	0.90	
Available phosphorus, %	0.50	0.50	0.45	0.45	

#### Table 2. Composition of basal diet<sup>1</sup>

<sup>1</sup>As-fed basis.

<sup>2</sup>Provided per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D<sub>3</sub>, 2,500 IU; vitamin E, 40 IU; vitamin K<sub>3</sub>, 5 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pyridoxine, 5 mg; pantothenic acid, 15 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 50  $\mu$ g; vitamin B<sub>12</sub>, 20  $\mu$ g.

<sup>3</sup>Provided per kilogram of complete diet: Cu, 5 mg; I, 1 mg, Co, 200 µg; Se, 150 µg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg. <sup>4</sup>Orafti IPS, Beneo Animal Nutrition, Oreye, Belgium. <sup>5</sup>*Enterococcus faecium* NCIMB 10415, (2 x 10<sup>10</sup> cfu/g) Cylactin ME20, DSM Nutritional Products, Basel, Switzerland.

		Treatments <sup>1</sup>			Statistics	
Item	NC	PC	S	SEM	P-value	
Egg Weight, g	59.46	59.48	59.49	0.10	0.995	
Hatchability, %	92.44	91.22	90.88	0.83	0.741	
Hatching Weight, g	41.38	42.56	41.90	0.36	0.428	
Liver Weight, g	1.05	1.04	1.01	0.02	0.671	
Heart Weight, g	0.32	0.34	0.33	0.01	0.295	
Yolk Weight, g	4.63	4.91	4.93	0.19	0.780	
Gizzard Weight, g	2.30	2.43	2.30	0.05	0.543	
Bursa Fabricius Weight, g	0.055	0.053	0.054	0.002	0.907	

**Table 3.** Effects of in ovo synbiotic administration on hatchability (%), hatching weight and internal organ and yolk weights on day of hatch.

<sup>1</sup>NC: Negative control, non-injected group; PC: Positive control, injected with 0.9% NaCI; S: Synbiotic group, injected with 0.5% inulin and 1 x 10<sup>6</sup> *Enterococcus faecium* NCIMB 10415 in 0.9% NaCI.

Dietary Treatments					Sta	Statistics	
Item	NC-Basal	S-Basal	NC-Syn	S-Syn	SEM	P-value	
0 to 7 d							
BWG (g)	100.21	102.45	96.89	92.60	1.54	0.114	
Feed intake (g)	138.14	133.43	128.86	126.14	2.36	0.299	
FCR	1.38	1.30	1.33	1.37	0.02	0.635	
0 to 14 d							
BWG (g)	348.31	353.73	344.32	326.53	5.00	0.251	
Feed intake (g)	530.37	506.00	508.31	482.16	4.28	0.124	
FCR	1.52	1.44	1.48	1.49	0.02	0.510	
0 to 21 d							
BWG (g)	796.76	793.16	768.89	742.39	10.12	0.202	
Feed intake (g)	1186.94	1148.00	1112.73	1098.44	11.61	0.388	
FCR	1.49	1.45	1.45	1.48	0.01	0.657	

**Table 4.** Effects of in ovo synbiotic administration and continued receiving in the diets on body weight gain, feed intake and feed conversion ratio of broiler chickens

<sup>1</sup>Data represent mean values of 7 replicates per treatment.

<sup>2</sup>NC-Basal: non-injected group fed basal diet; S-Basal: synbiotic injected group fed basal diet; NC-Syn: non-injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed; S-Syn: synbiotic injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed.



Dietary Treatments <sup>2</sup>					Statistics	
Item	NC-Basal	S-Basal	NC-Syn	S-Syn	SEM	P-value
Jejunum						
Villus height (µm)	790.50	837.12	775.94	866.91	15.09	0.127
Crypt depth (µm)	108.60 <sup>b</sup>	116.94 <sup>a</sup>	108.46 <sup>b</sup>	121.40 <sup>a</sup>	1.28	0.000
VH:CD ratio	7.27	7.16	7.15	7.14	0.10	0.969
Ileum						
Villus height (µm)	513.48 <sup>b</sup>	570.06 <sup>ab</sup>	564.43 <sup>ab</sup>	$583.70^{a}$	9.36	0.028
Crypt depth (µm)	100.02 <sup>b</sup>	110.97 <sup>a</sup>	105.97 <sup>ab</sup>	109.59 <sup>a</sup>	1.21	0.002
VH:CD ratio	5.20	5.14	5.24	5.32	0.06	0.754

**Table 5.** Effects of in ovo synbiotic administration and continued receiving in the diets on histomorphological parameters of the jejunum and ileum on d 21<sup>1</sup>

<sup>a, b</sup> Means with different superscripts in the same row are significantly different (P < 0.05).

<sup>1</sup>Data represent mean values of 7 replicates per treatment.

<sup>2</sup>NC-Basal: non-injected group fed basal diet; S-Basal: synbiotic injected group fed basal diet; NC-Syn: non-injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed; S-Syn: synbiotic injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed.

**Table 6.** Effects of in ovo synbiotic administration and continued receiving in the diets on the population of coliform and *Lactobacillus* in cecum ( $\log_{10}$  cfu/g) at d 21<sup>1</sup>

Dietary Treatments <sup>2</sup>			Sta	tistics		
Item	NC-Basal	S-Basal	NC-Syn	S-Syn	SEM	P-value
d 21						
Coliform	8.04 <sup>a</sup>	7.92 <sup>ab</sup>	7.75 <sup>b</sup>	7.75 <sup>b</sup>	0.03	0.000
Lactobacillus	8.74 <sup>c</sup>	9.04 <sup>b</sup>	9.14 <sup>a</sup>	9.21 <sup>a</sup>	0.04	0.000

<sup>a-c</sup> Means with different superscripts in the same row are significantly different (P < 0.05).

<sup>1</sup>Data represent mean values of 7 replicates per treatment.

<sup>2</sup>NC-Basal: non-injected group fed basal diet; S-Basal: synbiotic injected group fed basal diet; NC-Syn: non-injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed; S-Syn: synbiotic injected group fed 1% inulin and  $2 \times 10^9$  *Enterococcus faecium* cfu/kg feed.

# **POSTER PRESENTATIONS**



## P<sup>01</sup> The Effects of Preslaughter Shackling on Some Stress Parameters, Fear, and Behavioural Traits in Broilers

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#### Abstract

The aim of this study was to investigate the effects of preslaughter shackling durations on some stress parameters, fear reactions and behavioural traits in broilers. Day old 272 broiler chicks (Ross 308) were randomly allocated into 4 groups containing 34 birds (17 male, 17 female) for two replication. After 42 days regular rearing period, birds were exposed to 10 (control), 30, 60, and 120 s shackling durations. Results showed that heterophil to lymphocyte (h/l) ratio (1.39) at 120 s shackling group increased (P<0.01). It was revealed that shackling had increased blood glucose and cholesterole levels in all treatment groups. Results indicated that shackling duration has no significant effect on Tonic Immobility (TI) duration. The straightening up of the body, vocalisation, and wing flapping activities increased in due to increase in shackling duration. It was concluded that shackling duration over 60 s have negative effects on some stress parameters and behavioural traits in broilers, for that reason broilers should housed at lower preslughter shackling durations.

*Keywords:* Broiler, Shackling, Stress, Tonic immobility \*: This study was supported by ADU (The Project of Scientific Research, No: VTF 12-007)

## P<sup>02</sup> Effects of Early Feed Restriction on Some Performance and Reproductive Parameters in Japanese Quail (*Coturnix coturnix japonica*)

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#### Abstract

This experiment was conducted to investigate the effects of early feed restriction on some performance, production and hatchability traits in Japanese quail. Two weeks old birds were randomly allocated to three feed restriction groups with three replicates 7 males, 7 females. Body weight, live weight gain, feed conversion ratio were measured during growth period (between 2 to 5 week of age). In egg production period (from 6 to 15 week), body weight and age at first egg, egg production, fertility, hatchability, embryonic mortality, liveability, vitality and hatching chick weight were evaluated from replicates of one female and one male per treatment. In 3rd and 4th weeks of age, body weight was found higher *ad libitum* group than 15 and 30% restricted feed groups. In 5th week, average live weight gain of group *ad libitum*, 15 and 30% feed restriction, 5.18, 6.97 and 8.37 g, respectively. In 12 week of age, hatching chick weight was heavier for 30% feed restriction group than *ad libitum* group. Body weight and age at first egg, egg production, fertility, hatchability, embryonic mortality, liveability and vitality were unaffected by feed restriction. As a result, 15 and 30% restriction of *ad libitum* feed intake from 2 to 4 week of age without detrimentally affecting reproductive and hatchability parameters between 6 to 15 week of age.

Key Words: Feed restriction, growth performance, hatchability, quail



## P<sup>03</sup> Nutritive Value of *Camelina* Meal and It's Effects on Performance and Meat Quality in Broilers

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#### Summary

This review summarizes findings on the use of *Camelina* meal in diets of broilers. Various studies that were focused on the effect of camelina meal on growth performance and meat quality in broilers. The review summarizes the information of camelina meal's nutrient composition including crude nutrients, fatty acid composition and antinutritional factors, effects on broiler performance and meat quality. This data shows beneficial effect of *Camelina meal* in the production of broilers and some intersting functional aspects that might be interesting for nutritionists and the poultry companies.

#### Introduction

Poultry meat is a major source of animal protein considering that consumers' preferences for its' nutrient composition, convenient cooking and low cost. In Turkey, poultry meat consumption has reached approximately 19.3 kg/person/year (1). Polyunsaturated fatty acids are found abundantly in poultry meat and these fatty acids, especially n-3 fatty acids such as a linolenic acid, are considered as beneficial for human health. N-3 fatty acids must be obtained from the diet (2, 3). Bond et al. (4) indicated that the omega-3 content of poultry meat may be incremented with increasing the this fatty acid content of broiler diets.

Polyunsaturated fatty acids are found abundantly in poultry meat. These fatty acids, especially n-3 fatty acids such as linoleic acids are not synthesized in human body. Therefore, n-3 fatty acids must be present in human diets (2, 3). Broiler meat is a rich omega-3 source for humans. Increases in the level of omega-3 content in poultry diet has increased the concentration of this fatty acids in broiler meat as well (4).

*Camelina sativa*, which is known as false flax or German sesame of the Brassicaceae family, contains high concentrations of linolenic acid, and is an important alternative oilseed crop. It is native to Europe and to Asian areas. Camelina meal is a by-product that biodisel production process and could be used as an alternative n-3 fatty acid providing feedstuff to poultry diets (5, 6). Camelina meal includes some anti-nutritional factors and understanding the composition of these compounds in the meal is important to use it at the appropriate levels in the diet.

The aim of this paper was to examine the nutrient profile of camelina meal, effects on poultry meat quality and its potential use as an ingredient in poultry diets.

#### **Chemical Components**

Chemical composition of *Camelina* meal has been investigated to assess its value as a feedstuff. *Camelina* meal consists of approximately 35% crude protein, 13% oil, 10% fibre (Pekel et al., 2009). Chemical composition of the meal depends heavily on the oil extraction process and 4.9

% ether extract level has also been reported. Table 1 shows the analysis result obtained from camelina meal (7-9).

 Table 1. Chemical composition of Camelina meal

Parameters, %	Amount
DM	93.50
СР	35.20
Ether extract	4.90
Ash	6.50
Fiber	9.90
Neural detergent fiber (%)	41.80
Gross Energy (kcal/kg)	4822.00

#### Amino Acids

As a protein source *Camelina* meal's amino acid composition has also been investigated in order to ascertain its biological value. Despite having similar crude protein levels, *Camelina* meal's lysine and sulphur containing amino acid level is lower than the canola meal's. On proportional basis sulphur containing amino acid level of *Camelina* meal is higher than the soybean meal's sulphur containing amino acid level. However, lysine proportion in the soybean meal is higher than the *Camelina* meal's lysine proportion (10). Table 2 shows the essential amino acid composition of *Camelina* meal (8).

Essential amino acids	Amount
Arginine	2.86
Histidine	0.83
Isoleucine	1.25
Leucine	2.20
Lysine	1.59
Methionine	0.59
Phenylalanine	1.44
Threonine	1.34
Valine	1.75

Table 2. Essential amino acid composition (% of protein) of Camelina meal.

#### Fatty Acids

Fatty acid composition of the *Camelina* meal is unique in terms of the level of essential fatty acids it contains. High levels of the unsaturated and essential fatty acids in the *Camelina* meal make it different from most of the feedstuffs. Compared to soybean meal and canola meal, proportion of  $\alpha$ -linoleic and gadoleic acid in camelina meal is higher (10). Oil extracted from the camelina meal contains 29.6%  $\alpha$ -linoleic acid, 23.4% linoleic acid and 32.7% total monounsaturated fatty acids. Table 3 gives the fatty acid composition of camelina meal (11).



Table 5. 1 arty dela composition of Camerina	mear (70 of ether extract):	
Fatty acids	Amount	
Palmitic (16:0)	9.00	
Palmitoleic (16:1)	0.20	
Stearic (18:0)	2.50	
Oleic acid (18:1)	20.20	
Linoleic (18:2n-6)	23.40	
α-Linolenic (18:3n-3)	29.60	
Eicosenoic (20:1)	10.10	
Eicosadienoic (20:2)	1.40	
Eicosatrienoic (20:3)	1.20	
Erucic (22:1)	1.70	

Table 3. Fatty acid composition of *Camelina* meal (% of ether extract).

#### Antinutritional Factors in *Camelina* Meal

As a member of *Brassiacaceae* family, *Camelina* seeds are also expected to contain antinutritive compounds such as glucosinolates and sinapine. It has been suggested that concentration of antinutritional compounds in the seed is associated with the genotype of the plant. Glucosinolate level in *Camelina* seeds from different genotypes ranges between 15.2 and 24.6 mmol/kg (12). Aziza et al. (13) also reported similar level of glucosinolate level (24.4 mmol/kg) for Camelina meal as well. However, another study indicated (Table 4) higher level (34.4 mmol/kg) of glucosinolate for the Camelina meal (10). It has been suggested that Camelina meal contains higher concentration of glucosinolates than the seed itself. While seeds of Camelina contains 9-19 mmol/kg of glucosinolates, Camelina meal produced from the same type of seeds contains 14.5-23.4 mmol/kg glucosinolate (14).

 Table 4. Glucosinolate level in Camelina meal (mmol/kg).

Items	Amount
9-(methyl-sulfinyl) nonyl (glucoarabin)	9.04
10-(methyl-sulfinyl) decyl (glucocamelinin)	21.59
11-(methyl-sulfinyl) undecyl	3.80
Total glucosinolates	34.43

Sinapine has been detected in the different plants belonging to *Brassicaceae* family (15). Sinapine is the component which imparts bitter taste to oilseeds. Camelina seeds contain 4 mg/g sinapin (16). Sinapin content of Camelina meal has also been investigated and very different results (1.7-4.2 mg/g) have been reported depending on the location and growing season of the seed which is used for oil extraction (14). Condensed tannin level in Camelina meal is lower than the seed itself and reported between 1.0-2.4 mg/g depending on the sample (14). Camelina oil is reported to contain 2-4% erucic acid which is higher than the level in canola oil (5).

#### Effects of Camelina Meal on Broiler Performance

Camelina meal's effect on broiler performance has been studied (8). Camelina meal constituted 10% of the diet at the expense of soybean meal. Live weight of birds fed on camelina meal was reported to be lower than the birds in the control group. Reduced feed consumption was reported for the broilers which had 10% Camelina meal in their diets. Another study (9) has reported lower feed efficiency with the inclusion of camelina meal in the broiler diet during the first 21

days of the trial. However, feed efficiency in the grower period (22 - 42 days) were higher in the birds which were fed on Camelina meal. Reduction in the body weight gain of the birds have been reported at the different inclusion rates of Camelina meal in the diet.

Ryhanen et al. (7) reported retarded growth in broilers fed with Camelina meal. Study has also shown that inclusion of Camelina meal in the diet reduced feed intake during the early stage of growth (1-14 days). Feed efficiency has been reduced in the broilers fed on diets which include Camelina meal.

#### Effects of Camelina Meal on Broilers Meat Quality

Inclusion of *Camelina* meal in the poultry diets has been investigated in order to ascertain its possible effects on different meat characteristics (9, 13). Incorporation of *Camelina* meal into broiler diets at the level of 10% has been reported to increase the antioxidant capacity of breast and thigh meat. Tocopherol level of the thigh meat has also been increased with the inclusion of *Camelina* meal. However, same tendency for tocopherol level was not observed in the breast meat. There was not any change in the meat's phenolic compounds concentration with the inclusion of *Camelina* meal.

Use of *Camelina* meal in the broiler diets did not change the fat content of breast or thigh muscle (9). However, effects on the fatty acid composition of the meat has been observed. Concentration of  $\alpha$ -Linolenic acid in the breast meat, thigh meat and liver has been increased with the incorporation of *Camelina* meal into diet. Ratio of n-6:n-3 in muscles were significantly reduced because of the increased n-3 level in the muscles.

Ryhanen et al. (7) reported that inclusion of different levels of camelina meal in broiler diet has linearly increased omega-3 fatty acid concentrations in the broiler meat. This results confirm the findings of other studies (9, 13). Saturated fatty acids level in the meat has been reduced with the inclusion of 10% *Camelina* meal to diet. Erucic acid content of the broiler meat was low in the broilers fed with *Camelina* meal so meat produced from those animals is safe for human consumption. However, gondoic acid content of the meat has been reported to increase with the use of *Camelina* meal in diets.

#### Conclusion

Global consumption of the broiler meat has been increased drastically in the last decade. Broiler meat producers seek new raw materials in order to reduce their production costs. A by-product of biofuel production, *Camelina sativa* meal, could be incorporated into broiler diets as a low cost alternative. However, studies on its nutrients availability in broilers need to be done. Further studies on the assessment of individual amino acid availability of *Camelina* meal would provide more information to nutritionist formulating broiler diets. Besides, use of *Camelina* meal in broiler diets would change the nutritional composition of broiler meat. Studies show that inclusion of *Camelina* meal to broiler diets would have a potential to produce functional meat production. It has been suggested that glucosinolate content of *Camelina* meal have a detrimental effect on the performance of broilers. Glucosinolate concentration in the meal has been measured in a very broad range depending on the location and the genotype of the product. So it would be beneficial to carry out researches in order to reduce anti-nutritional compound content of the seed by



selecting suitable genotypes. Reduction in the level of anti-nutrient content of the *Camelina* meal might increase its value as a feedstuff in broiler nutrition.

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## P<sup>64</sup> Usage of Herbal (Solanum glaucophyllum) 1.25-dihydroxycholecalciferol (1.25(OH)2D3) in Broiler Breeder Diets

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#### Abstract

The aim of this study was to determine effects of herbal (*Solanum glaucophyllum*) 1.25-dihydroxycholecalciferol  $(1.25(OH)_2D_3)$  supplementation on the egg weight, eggshell quality and hatching results in broiler breeders. The experiment was performed at breeding hen house containing 40000 broiler breeders (49 weeks old) which belongs to Hastavuk A.Ş. The diets used in experiment also were prepared at the feed company of Hastavuk A.Ş. During experiment, broiler breeders in control group were fed with Broiler Breeder Diet 1(49-53 weeks) and Broiler Breeder Diet 2 (54-59 weeks). Broiler breeders in treatment group were fed diet containing 10 ppm of herbal  $1.25(OH)_2D_3$  differently from control group. A significant improvement was determined at 59<sup>th</sup> week in eggshell quality ( P<0.001), eggshell breaking strength (P<0.05), cracked egg ratio ( P<0.05) in treatment group supplemented with  $1.25(OH)_2D_3$ . However, there was no significant difference between control and treatment group in egg weight and hatching results. It was concluded that the supplementation of *Solanum glaucophyllum* sourced  $1.25(OH)_2D_3$  to the diets of aged broiler breeders may be effective and its effect can be demonstrated on the hatching results in long-term researh.

**Key Words:** Broiler Breeder, Solanum glaucophyllum, 1.25-dihydroxycholecalciferol, eggshell quality, hatcing results.



## P<sup>05</sup> Effect of Eugenol and Sauce on Broiler Baguettes Stored at +4 °C Contaminated with *Salmonella* spp.

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 <sup>3</sup>Tunceli University, Faculty of Fisheries, Tunceli, Turkey

#### Abstract

In this study, it was investigated antibacterial effect of sauce and eugenolon broiler baguettes contaminated with Salmonella strains. Prepared for this purpose; contaminated but the sauce does not contain (group 1), contaminated and contain sauce (group 2), and contaminated-sauce and eugenol containing 0.3% (group 3), and contaminated-sauce and eugenol containing 0.5% (group 4) baguettes are packed in containers polyethylene cap +4°C were stored at 5 days. Microbiologycal analyses in order to presence of *Salmonella* spp. were done at the 0th, 2nd, 3rd and 5th days. The results of study 1 and 2 groups, depending on the storage period decreased slightly, depending on the storage period of the increased in the other groups were determined. Most of the storage period decreased on the day of 5th 0.40 log10cfu/g and 1 group, the 5th of at least 0.19 log10 decrease in cfu/g and 2nd group, 3rd and 4th groups established light level increased. In conclusion, it can be stated that sauce and different eugenole concentrations have not got a significant effect on the broiler baguettes contaminated and 4with *Salmonella* strains.

## P<sup><u>06</u></sup> Decontamination of Poultry Carcass with Chemical Methods

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#### Abstract

Food poisoning from poultry meat can be reduced by chemical substance applications. Application of chemical substance on carcass limits the growth of pathogen and saprophytic microorganisms and reduces their numbers. In recent years, there has been an increasing interest in effective decontamination applications due to the fact that animals may be the origin of foodborne pathogens. Decontamination considerably prevents contamination that may result from the process of slaughter.



## P<sup>07</sup> Evaluation of Single Application of Cetylpyridinium Chloride (CPC) and Tri-Sodium Phosphate (TSF) on Broiler Carcass Contaminated with *L. Monocytogenes*\*

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#### Abstract

This study was conducted with the aim of evaluating the effects of cetylpyridinium chloride (CPC) and tri-sodium phosphate (TSF) on *L. monocytogenes* on broiler carcass. Broiler carcasses were experimentally contaminated by mixing with 10 strains of *L. monocytogenes*. Then by decontaminating with 0.2% and 0.4% CTC and 8% and 12% TSF at 20°C for 15 minutes, the effects of those materials on lives of pathogens were determined. It was determined that there were significant differences between all of pre-decontamination and post-decontamination solutions used in terms of total numbers of bacteria (P<0.05). The city water is used as control group. Plantings were made from rinsing water of decontaminated carcasses. In the scope of this study; by holding the carcasses in 8% and 12% TSP and 0.2% and 0.4% CPC, the obtained decreases for *L. monocytogenes* are 2.09  $\log_{10}$  kob/ml and 2.38  $\log_{10}$  kob/ml and 3.89  $\log_{10}$  kob/ml we 5.04  $\log_{10}$  kob/ml, respectively. As a result, it was determined that 0.4% CPC is more effective on *L. monocytogenes* on broiler carcasses and it is followed by 0.2% CPC. It was revealed that CPC is more effective on eliminating *L. monocytogenes* on broiler carcasses than TSP.

\*: This study was supported by FUBAB (The Project of Scientific Research, No: 1314) and summarised by PhD thesis of first author

### P<sup>08</sup> Insects as an Alternative Feed Sources

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#### Abstract

Insecta belongs to arthropoda phylum. Life stages of insecta are classified in two groups; semimetamorphosis and complete metamorphosis. Hatched larvae of hemimetabolous insects is not similar with adult form and after a complete metamorphosis they turn in to adult forms. Hemimetabolous insects pass through a larval stage than enter an inactive state called pupa and emerge as adults. There are 1900 incest species and forms used in human and/or animal nutrition. World-wide population increases in recent years which results with an increased demand for animal based protien sources and encouraged scientists to find alternative feedstuffs. Results obtained from experiments show that edible insects can be a promising alternative feedstuff and high quality protein source in animal nutrition.



## P<sup>09</sup> Effects of Chitosanoligosaccharide Supplementation to Broiler Ration on Growth Performance, Carcass Traits and Visceral Organs Weight

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#### Abstract

This research was conducted to determine effects of chitosan oligosaccharide supplementation on broiler rations, growth performance, carcass yield traits and visceral organs weight. In this trial, a total of 375 one day old Ross 308 broiler male chicks were used. The chicks were randomly divided into 3 main groups (Control, Trial I and Trial II), each involving 125 chicks, each main group divided into 5 replicate subgroups each containing 25 chicks. Chicks were fed with starter diet for 1-14th days, grower diet for 15th-28th days and finisher diets for 29th-42th days. Control group was fed these diets, no additive, Trial I and II were fed with the same diet but additionally 50 and 100 mg/kg chitosan oligosaccharide supplemented their diet, respectively. There were no statistical differences on the final average body weight, body weight gain, feed consumption and feed conversion ratio amoung the groups. Cold carcass yields of the Trial I and II groups were significantly higher than the Control group (P < 0.05). There were no differences about the breast meat ratio amoung the groups. Thigh and wing ratio were significantly higher in the Trial I and Trial II than Control. Dorsa weight, back weight and abdominal fat ratio did not differ among the groups. Mesenterial fat ratio in Trial I was significantly higher than the Control group (P<0.05). Liver ratio in the Trial I and II were significantly lower than the Control (P<0.01). In conclusion: 50 and 100 mg/kg chitosan oligosaccharide supplementation to the broiler diet did not change growth performance; increased breast, thigh and wing ratio; but reduced mesenterial fat ratio, in this way it improved carcass yield, hereby it is concluded that it might be use above doses as an natural feed additive

\*: This study is summarised by PhD thesis of first author

## P<sup>10</sup> Evaluation of Feed Grade Enzymes by Using *In Vitro* Digestive Tract Model

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#### Abstract

Enzymes are widely used as feed additives in poultry rearing. It is not easy to determine the performance of commercial enzymes by using given enzyme activities. The main reason is that specific substrates and conditions (pH, temperature) used by activity measurements do rarely represent gastro intestinal system of poultry. An in vitro simulation system for GI tract of poultry is developed. Parameters like pH, mechanical forces, temperature and duration time can be set in this laboratory device. Feed rations are mixed with commercial enzymes by recommended doses and the digestion is simulated. At the end of the digestion elaborated carbohydrates, proteins and phosphate are analysed in comparison to control without enzyme addition. This system is used to determine and compare the performance of commercial enzymes as well to design new feed enzyme combinations.

Key words: poultry GI tract simulation, feed additive enzymes



## P<sup>11</sup> Effects of Carrot Fiber and Carrageenan Scale on Chemical, Technological and Textural Properties of Chicken Hamburgers

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#### Abstract

This study aims to analysis the effects of carrot fiber and carrageenan scale on chemical, technological and textural properties of chicken hamburgers. These effects are modelled by the application of the Central Composite Design model in accordance with the Respone Surpose Methodology.

In the this study it was made use of 64 week spent hen meat as raw material, additionally spices, lamb tail fat, carrot fiber (%0-2), and carrageenan (%0-1) combinations are chosen as ingredients for the chicken hamburger.

Various analysis on sensory, chemical, technological and textural properties of these chicken hamburger were done. Among the chemical properties of carrot fiber as additive in the chicken hamburger production, moisture; for texture properties, cutting force, cutting task, cutting energy, hardness and stickiness; and in the case of technological properties, diameter decrease and thickness increase are found as significant effects with respect to sensory analysis and color parameters (L\*,a\*.b\*) effects.

It is observed that the utilized carrageenan scale a\* and b\* color values are effective on the reduction of diameter and on the thickness increase.

Key words: Spent Hen, Chicken hamburger, Carrot fiber, Carrageenan, Response Surface Methodology

## P<sup>12</sup> Effects of Dietary Olive Leaf Supplemention to Broiler Diets on Performance, Some Blood Parameters and Intestinal Microflora\*

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#### Abstract

The study was conducted to investigate the effects of dietary olive leaf supplemention to broiler diets on performance, some blood parameters and intestinal microflora. In trial, three hundred and twenty one-day-old mixed sex broiler chicks (Ross 308) were used and randomly assigned to four treatment groups (80 birds/group), each consisting five replicates (16 birds/replicate). The 4 treatment groups formed in trial were fed diets; unsupplemented with olive leaf (control) or supplemented with 5, 10 and 20 g/kg olive leaf, respectively. The trial was maintained 6 weeks. The effect of treatment was determined for body weight at 28 and 35 days of age, for body weight gain and feed intake from 21 to 28 days of trial at significant level (P < 0.05). The dietary supplementation of 5 g/kg olive leaf caused to significant increase in body weight and body weight gain, and also 5 and 20 g/kg olive leaf supplementation caused to significant increase in feed intake compared to the control and other treatment groups at mentioned periods. The effect of treatment was not at significant level on feed conversion ratio and livability (P>0.05). No significant (P>0.05) differences were determined on performance criteria at 0-21, 22-42 and 0-42 days of the trail. The carcass parameters (carcass yield, relative weight of thigh and breast, and abdominal fat), organ (liver, spleen, bursa of fabricius, empty gizzard weight) and intestinal weights (small and large intestines, caecum) and blood parameters (AST, AST, albumin, total protein, triglycerides, total cholesterol, HDL and LDL) were not affected by dietary olive leaf supplementation (P>0.05). While ileal Lactobacillus spp. and Escherichia coli counts were significantly (P<0.05) affected by treatment, Clostridium perfringens and Staphylococcus *aureus* counts were not affected (P>0.05) by treatment. The dietary supplementation of 10 g/kg and 20 g/kg olive leaf significantly (P < 0.05) increased *Lactobacillus* spp. count and decreased Escherichia coli count in ileum as compared to the control group. The absence of Salmonella spp. was determined in ileum of birds from each treatment groups. As a result, the supplementation of 10 and 20 g/kg olive leaf to broiler diets improved intestinal microflora, without affecting the performance and the other examined parameters.

**Key Words**: Broiler, olive leaf, antimicrobial, performance, blood parameters. \*: This study is summarised by master thesis of first author


# P<sup>13</sup> Chicken Meat Consumption Trends of Ege University Students

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#### Abstract

The aim of this survey was to determine chicken meat consumption trends of Ege University students The questions were asked to 200 students from 10 faculty (20 students from each). The male to female ratio was 83:117 in the study. The majority of female students prefer to consume breakfast and lunch regularly, on the other hand the majority of male students do not prefer having breakfast and lunch regularly. It is determined that, male and female students prefer chicken meat and products when they have meals or fast-food consumption. The chicken meat seems to have an important place within the eating habits of the students. Unsubstantiated statements made on antibiotic and hormones affect the chicken meat consumption of students.

# P<sup>14</sup> Factors Affecting Performance of Broilers in Integrated Broiler Operation

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#### Abstract

This study aimed to investigate the factors affecting performance of broilers in an integrated broiler operation. For this purpose, 3 years data of broiler contracted producers were evaluated. Effects of breeder age (28-38, 39-54, 55-77 week), and season (winter, spring, summer, fall) on slaughter weight, feed conversion ratio, and mortality rate were evaluated. The effects of transport distance (<80, 81-160, >161 km) and season on mortalities during transportation from poultry house to slaughter were also investigated. The results showed that, slaughter weight increased with increasing in breeder age. Both of breeder age and rearing season had a significant effect on mortality rate. Broilers from old breeders showed higher mortality rate than those from middle- and young- aged breeders. The highest mortality rate was observed in summer and fall whereas it was lower in winter. Although deads on road increased with the increase in transport distance from farm to slaughter house, the interaction between transport distance and season showed that this effect was only significant in fall.

Keywords: Breeder age, season, transport distance, performance



# P<sup>15</sup> Evaluation of Health, Economic and Performance Status of Feed Additives Replacing the Antibiotic Growth Promoters After the Ban in EU and Turkey

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#### Abstract

Today the world population is approximately 7.3 billion and it is increasing rapidly. One of the main needs of the increasing population is the consumption of sufficient and safe food. However, safe food is hard to find and it is very expensive, particularly in underdeveloped and developing countries. In the countries in question, diseases caused by malnutrition are very common. In order to tackle this problem, alternatives have been sought for red meat, which is expensive and an important source of protein, and as a result of this, the production and consumption of poultry meat have emerged. Considering its consumption per capita, poultry meat is one of the most preferred products of animal origin for its low fat content, in addition to being cheaper compared to red meat. Other advantages to be mentioned here are its production volume and wide range of derived products. It is estimated that world poultry meat production was over 100 million metric tonnes in 2013 and 90 million metric tonnes of this amount was chicken meat. Turkey is becoming a more important actor in the area of poultry meat production. Furthermore, Turkish poultry meat production was 1.923.000 metric tonnes and domestic consumption of poultry meat per capita was 20,53 kg in 2013. Lead by its poultry integrations, Turkey has increased its exports and production of poultry meat for the foreign markets in the last period, as well as production of poultry meat placed on the domestic market. In 2013, Turkish poultry meat export was 395.694 metric tonnes. Using this data, it is important to increase and support poultry meat production in a planned manner in order to balance the distribution of poultry meat on the domestic market, increase the number of countries to whom we export and improve our ranking. In this review which has been prepared for the abovementioned purpose, our aim was to investigate, with regard to certain criteria, the use of feed additives, which are becoming more important in the preparation of diets after the use of antibiotics for growth promoting purposes was banned in the EU and in Turkey in 2006.

Keywords: antibiotics, feed additives, poultry production, economic analysis

# P<sup>16</sup> Effects of Breeding Age and Energy Restriction on Fattening Performance and Plasma Leptin Levels in Broilers

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#### Abstract

In this research, the effects of breeding age and energy restriction on fattening performance and plasma leptin levels of broiler were investigated. This goal 504 male chicks, obtained from young (27 wk), middle aged (38 wk) and old aged (60 wk) broiler breeders were used. Each age group was divided (energy restriction and normal feeding) into two groups. Energy restriction groups were fed between 7-14 days with energy-reduced diet. During the experiment body weight, body weight gain, feed consumption and feed conversion ratio were measured weekly and also plasma leptin level was determined. Results showed that breeder age affected body weight (P<0,001), total body weight gain (P<0,001) and total feed consumption (P<0,001). Energy restriction affected the total feed consumption (P<0,05) and total feed conversion ratio (P<0,01). Leptin level decreased with increasing age.

Keywords: Broiler; Energy restriction; Breeder age; Fattening Performance; Leptin.



# P<sup>17</sup> Hygine Application and It's Evaluation in Broiler Production

## Özlem Varol Avcılar

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### Abstract

Biosecurity is a concept that has important economic, social, ecological and health related dimensions. The most common definition of the biosecurity health plan or measures designed to protect a population from transmissible infections agents. Poultry health management is the emerging issue along with bio-security measure. Biosecurity measures evaluated survey metod and surveillance system. The objective of this study is to emphasize importance of biosecurity in broiler farms.

Keys: Biosecurity, broiler, health

# P<sup>18</sup> Foot Pad Dermatitis in Broilers and Turkeys

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### Abstract

Pododermatitis plantar metatarsal and plantar in foot deep a condition characterized by inflammatory changes. Ouse to severe ulcer lesions vary from hyperkeratosis and pigmentation disorders. Dermatitis soles (plantar pododermatitis) (foot pad dermatitis, FPD) metatarsals and phalanges lesions are commonly seen in broilers and turkeys have high body weight. What are the causes of FPD complex and multifactorial. Studies of broilers and turkeys where it is important for the FPD incidence growing conditions and wherein the feed program, the backing material, the depth of the pad and the wet mat has been reported to cause contact dermatitis. Soles of the feet and to be multifactorial dermatitis broiler until the formation is common in turkeys and broiler producers must therefore exercised due diligence in raising turkeys.



# P<sup>19</sup> Does the Way for Exporting Poultry Meat to EU Make Zoning and Compartmentalization to Overcome the Challenges ?

#### Şebnem Gürbüz

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#### Abstract

As is known for the exportation of animal products to the European Union (EU), it is necessary that the country should produce at least equivalent condition with the relevant EU standards in the field of animal health and public health. Since the early 2000s, Turkey has put forward efforts for the exportation of poultry meat and products to the EU and has been audited several times by the Food and Veterinary Office of DG Health and Consumers. The most important obstacle in front of the Turkish origin poultry meat and product to access the EU market is the Newcastle disease, which is endemic in Turkey. Having free from diseases in entire territory is highly difficult, World Animal Health Organization has published guidance and standards for establishing zoning and compartmentalization, free from animal diseases, pave the way for fighting against the diseases and continuation of trade. Due to eradication of poultry disease in entire territory, such as Newcastle, is highly costly and time consuming, it is necessary to establish a disease free zone or compartment in order to overcoming the obstacles regarding to export of poultry meat to the EU. On this matter, the EU is leaning towards accepting zoning rather than compartmentalization.

# P<sup>20</sup> Effects of Varying Dietary Valine Amino Acid Levels on Broiler Performance and Immune System\*

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#### Abstract

This study was conducted to investigate the use of valine, which is an essential amino acid in broiler diets, and to examine the effect on broiler performance (feed consumption, body weight, body weight gain, feed conversion ratio - FCR ), carcass yield, mortality, weights of immune system organs (thymus, bursa Fabricius and spleen) and vaccination antibody titers with the use of varying levels in different feeding periods. A total of 245 1-day-old male Ross 308 broiler chicks were used as the animal material in the study. Five dietary treatment groups (Control, D,,  $D_2$ ,  $D_3$  and  $D_4$ ) were arranged. Diets were based on corn, soybean meal and nut meal. Feed and water intake was ad libitum. The valine levels of the control group were % 0,9 for 0-21 days and % 0,82 for 21-42 days as recommended by NRC (1994) in ratio. The valine levels of D<sub>1</sub> ve D<sub>2</sub> groups were % 0,9 for 0-21 days as notified by NRC (1994) and % 0,92 and % 1,02 valine upper value of % 0,1 and % 0,2 recommended by NRC (1994) for 21-42 days. The value levels of D, ve  $D_4$  groups were % 1 and % 1,1 valine upper value of % 0,1 ve % 0,2 recommended by NRC (1994) for 0-21 days % 0.82 for 21-42 days as recommended by NRC (1994). Use of different dietary valine levels in different feeding periods did not create any statistical difference except numerical differences on feed consumption, body weight, body weight gain, feed conversion ratio - FCR, carcass yield and mortality rate (P>0.05) when all study periods were taken into account. Spleen and bursa Fabricius were not observed to be affected by various dietary valine levels (P>0.05) when periodic development of immune system and primary lymphoid organs were evaluated. But there was significant statistical difference in thymus development on both 0-21 days and 21-42 days in groups fed with ratio containing % 1 value level (P < 0.05). In terms of humoral immunity, although the highest degree of vaccination antibody titers were achieved in groups fed with a ratio containing % 1,1 valine level, this was not considered to be statistically significant. As a result of this study, optimal value of value in broiler ratios were evaluated to be % 1 for 0-21 days in contrast to NRC (1994) recommendations and % 0,82 for 21-42 days as recommended by NRC (1994).

Key Words: Broiler, Valine, Performance, Immune System, Newcastle

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# P<sup>21</sup> Poultry Report for East Part(Malatya, Elazığ, Bingöl, Tunceli) of Turkey

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### Abstract

Agriculture is mentioned a number of activities including; the production of vegetable and animal products, improving their quality and efficiency, the preservation of these products in appropriate circumstances, be processed and evaluated, and marketing. Literally, agriculture, which is a broad concept that can be roughly divided into three sub-sectors:

- Crop production
- Animal production
- Agricultural products and technology

In this study, "Poultry" group were examined in TRB1 Region (Malatya, Elazığ, Bingöl, Tunceli). Poultry group represents to grow chicken, turkey, goose, duck, ostrich, partridge, pheasant and quail enters various bird species for hobby or commercial purposes. Since the economic value of chickens are higher, raised and widely consumed for their meat and eggs. Ducks and other poultry breeding geese done as a hobby, it is excluded in this study.

In fact, eggs and broiler production is observed, to be considered as a sector in its own in the world and in our country. This production also contributes to the development of construction materials and tools, such as feed, cage, waterers and feeder, vaccines and also of the pharmaceutical industry.

Poultry Industry is developing since the productivity can be satisfied with less effort and maintainanace. That sector is raising in rural areas and having an important role for providing the need of protein for the country with their eggs and meat. Red meat and white meat can be substituted, especially more of the population is in low-income families, and they meet the protein requirements with white meat.

In this study, the poultry sector TRB1 has been examined by compiling surveys and scanning thesis work carried out in this region. The quantitative data of the sector has been compiled, the trend of the industry shown, and solutions of issues has been developed of this region.

# P<sup>22</sup> Variation in Breast Meat Color and Its Relationship with Meat Quality in Broilers Slaughtered in Turkey

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#### Abstract

The aim of this study was to investigate breast meat quality, variation in breast meat colour and its relationship with meat quality in broilers slaughtered in Turkey. For this purpose, a total of 1844 breast meat fillets obtained from Ross broilers were used to determine ultimate pH (pH<sub>24</sub>), colour features [Lightness (L \*), redness (a \*) and yellowness (b \*)], thawing and cooking losses water holding capacity and texture. All breast meats were classified as "pale" (L\*>54) or "normal" (48<L\*<53) and meat quality parameters were evaluated for each classification taking into consideration sex effect. The 74.6% of the all fillets was pale. Pale breast meats had higher a \* and texture with lower cooking loss and water holding capacity. Higher pH<sub>24</sub> values (5.93 ± 0.01) were obtained for normal breast meats compare to pale breast (5.76 ± 0.01). These results indicated a large variation in the meat lightness which was depend on the rate of muscle pH fall would affect water holding capacity and functional properties of breast meat.

Key words: Broiler, meat colur, meat quality, PSE



# P<sup>23</sup> Abdominal Fat Deposition in Broilers

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#### Abstract

As a result of 60 years of genetic selection of broilers, fast growing is paralleled with the unwanted increase of abdominal fat. Besides being an economic loss for the producer, excess fat also negatively influences diet utilisation and post-slaughter processing. One of the main aims in poultry production is to increase the amount of consumable meat while reducing the accumulation of abdominal fat. At the same time, a certain amount of carcass fat is desirable for better flavour and palatability. A lot of time, effort and a great amount of money have been spent on researching ways to reduce fat build-up. The present paper reviews the studies and results reported on the topic of abdominal fat deposition and on the genetic, gender, age, diet and environmental factors influencing the build-up of abdominal fat.

Key Words: Broiler chicken, abdominal fat

# P<sup>24</sup> Changes in Poultry Litter During Composting Period

### Gökhan Çaycı<sup>1</sup>, <u>Cağla Temiz<sup>1</sup></u>, Sonay Sözüdoğru Ok<sup>1</sup>, Gamze Depel<sup>2</sup>

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#### Abstract

In this research, composting of broiler manure, its production is about to 2,1 million tons per year in Turkey, in small scale aerated conditions and determination of changes in temperature and some plant nutrients contents during this period were aimed. Fresh broiler manure with rice hull as bedding material was received from a broiler house in the province of Bolu. At the beginning C/N ratio in compost pile was purposed as 25 in order to accelerate composting. For this reason, one hundred kg rice hull was added to eighty kg fresh broiler manure, and initial moisture of compost pile was brought to 50% as basis wet weight. Compost pile was placed to the composting bin afterwards pile was periodically turned and moistened different times. Composting processes was terminated nearly at the end of three months as the stability for temperature in compost pile was established. Composite samples were randomly taken from compost pile at initial, aeration days and at the final period. Total N, P, K, S, Ca, Mg, organic carbon and humic +fulvic acids were determined in these samples. As Initial and final data in compost pile were considered, total organic carbon, nitrogen and sulphur losses were about 41%, 34% and 42%, respectively. On the other hand, increases in total phosphorous, potassium, calcium, magnesium and humic+fulvic acid contents were determined as the rates of 46%, 11%, 44%, 53% and 18%, respectively.



# P<sup>25</sup> The Effect of Sex on Some Welfare Indicators Measured Either on Farm or Processing Plants in Commercial Turkeys

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#### Abstract

In this study, the effect of sex on some welfare indicators measured either on farm or processing plant in commercial turkey stocks has been investigated. A week before slaughter age (13 wk for females and 16 wk for males) 3 male and 3 female stocks were visited and an average of 100 sampled birds were examined for gait and pecking related damages on body parts, and 50 sampled birds were examined for foot pad dermatitis, hock burn, dirtiness and irritation on breast. Except with gait scoring, all the measures were observed and scored at processing plant in the same stocks. In addition to these observations bruising was recorded and alterations in breast skin were differentiated as breast blister and breast button (dermatitis). Distribution of males and females among the scores for each indicator were investigated by Chi-square and sex effect on scores has been analyzed by one way Wilcoxon/Kruskal-Wallis. Proportion of foot pad dermatitis and other welfare problems in female turkeys were higher ( $P \le 0.05$ ) than those in males. These findings were compatible to those recently reported by European survey studies in which higher foot pad dermatitis and other welfare problems in females were associated with the higher stocking density (bird/ per square meter) as compared to the males.

Key Words: turkey, welfare, sex effect, foot pad dermatitis

# P<sup>26</sup> Role of Poultry Nutrition in Preventing the Environment Pollution Related to Poultry Production

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#### Abstract

Environmental pollution is an inevitable concern resulted from intensive animal production. Animal nutrition plays a unique role in alleviating and protecting of environmental pollution in relevant factors. Much of the concern for pollution from poultry production is wastes from manure. There are a lot of measurements to prevent environmental pollution resulted from manure involving nitrogen, phosphorus, dry matter and odour. Main objective is improve feed nutrient efficiency.



# P<sup>27</sup> Evaluation of Poultry By Products as Protein Hydrolysates

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#### Abstract

The poultry meat consumption and production in worldwide is going up increasingly. The total poultry production reported in the world is 100 million tons. The biggest producer countries reported in the world are United States, China and Brasil respectively. Beside this big poultry production, a thousands tons of poultry viscera, feet, head, bone and blood are also produced as organic poultry by-products. Poultry by-products are generally converted to animal feed and organic fertilizer but espacially after the BSE (Bovine Spongiform Encephalopathy) outbreak, regarding the conventional convertion methods of animal by-products to animal feeds, the new regulations and methods has come up. Otherside, it is declared that few benefits are getting from this wast meterials with this conventional methods, but it is possible also to make added value products. Because of that reason, the new researches and radical changes regarding the alternative converting methods of poultry by-products are emphasized nowadays.

**Keywords:** Poultry by-products, protein hydrolysates, protein isolates, chicken gelatine, bioresources.

# P<sup>28</sup> Economic Impact of Ectoparasites in Poultry Production Systems

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#### Abstract

There are several types of arthropods that constitute the major ectoparasites of poultry primarily lice, bugs, fleas, mites and ticks. They occur in environments with poor hygiene and where insecticides and/ or acaricides rarely used to control the parasites. Ectoparasites cause weight loss, irritation by sucking blood and reduce the egg yield and other production performances. Costs for treatment and control measures also led to economic losses in poultry production systems. The degree and type of infestation is markedly influenced by the production method.

#### Introduction

Poultry production is becoming one of the most higly developed segments of food animal production globally. Among the disease of poultry, ectoparasites have a major impact on husbandry, productivity and welfare (Tolossa and Tafesse, 2013). Ectoparasites of poultry live on the skin or penetrate within the skin or even in to the air sacs and some live under the feathers. These ectoparasites feding on dead cells of skin, tissue fluids and feathers, while other suck blood (Urquhart, 1987).

Ectoparasites cause intense pain, irritation, slow weight gain, decreased egg production, and general poor and ill health (Kaufman, 1996). Also, they may act as vectors for pathogenic agents, resulting in serious diseases in chickens and humans (Arends, 2003). There is inappropriate housing and lack of considerable pest control efforts taken for these parasites (Mungube et al., 2008).

#### Progress

Being permanent ectoparasites lice are an important ectoparasites of poultry which cause ill health in poultry and causing irritation to the birds, which adversely affects the economical production of poultry. It was found that *Menacanthus stramineus, Menopon gallinae, Goniodes gigas, Goniocotes gallinae, Lipeurus lawrensis tropicalis, Lipeurus caponis* and *Cuclogaster heterographus* were the most commonly infesting lice of poultry (Muhammad et al., 2003; Bala et al., 2011). Lice infestation causes weight loss at the rate of about 711 gms per birds and decrease the egg yield at the rate of about 66 egg per bird in a year (El-Kifl et al., 1973) and lameness is associated with heavy lice infestation (Okaeme, 1989). Some researchers reported that lice infestation decrease in egg production in the 11 % (Edgar and King, 1950) to 15 % (Glees and Raun, 1959) range. Other researcher reported that the average hen weight had decreased by about 85, 300, and 450 grams in infested hens with *M. stramineus* (Devaney, 1976).

Dermanyssus gallinae, Ornithonyssus (Liponyssus) sylviarum and Ornithonyssus bursa can be serious pests due to their blood-sucking habits. Very large populations of Dermanyssus gallinae (the red mite) can build up rapidly in poultry houses. Dermanyssus gallinae, sucks



blood from poultry at night and remains secluded during the day and in cracks and crevices. When numerous, weight gains and egg production can be reduced. Besides, especially the mites also can be pests of people working with or around the hens (Jordan, 1990; Kettle, 1995; Ruff, 1999; Sparagano et al., 2009). Unlike *Dermanyssus, Ornithonyssus* never leaves the host and in heavy infestations causes blackening of feathers due to excreta and dark egg masses. In heavy infestations, birds are restless and lose weight from irritation, egg production may be reduced, and there may be severe anaemia (Jordan, 1990; Kettle, 1995; Ruff, 1999). Mullens et al. (2009) reported that unmitigated infestations with *O. sylviarum* overall reduced egg production (2.1-4.0 %), individual egg weights (0.5-2.2 %), and feed conversion efficiency (5.7 %), causing a profit reduction of \$0.07-0.10 per hen for a 10-week period. Mites can also annoy egg handlers and other persons (Jordan, 1990; Kettle, 1995; Ruff, 1999).

Burrowing mites of the genus *Cnemidocoptes* may cause feather loss, (depluming itch mite *C.gallinae*) or excessive scaliness of the skin, leading to thickening an deven deformation of the legs (scaly leg mite, *C. mutans*) (Jordan, 1990). Infestations of *C. mutans* develops slowly over many months while the bird loses its appetite and wastes away (Kattle, 1995). Other mites that may cause problems include some invasive species, which may be found in airsacs (*Cytodites nudus*) or subcutaneously (*Laminosoptes cysticola*) (Jordan, 1990). Small numbers of *Cytodites nudus* have no noticeable effect on the host, but in vast numbers they may cause death. *Laminosoptes cysticola* bring about the formation of nodules which become calcified on the death of the the mite, and reduce the market value of the carcass (Kettle, 1995).

The soft tick, *Argas persicus* which is blood feed for short periods at night, spending most of their time off the host, hidden in cracks and crevis. Apart from anaemia, anorexia, weight loss and depressed egg output (Kettle, 1995; Ruff, 1999).

Severel species of flea have been reported from poultry. The sticktight flea, *Echidnophaga gallinacea* is unique among poultry fleas as the mouthparts are deeply embedded in the skin making the adults sessile. The flea is occasionally found in the poultry house (Jordan, 1990; Ruff, 1999).

The common bedbug, *Cimex lectularius* occasionally attacks poultry. However, large numbers of this parasite can cause severe effects on production. Although many Diptera will feed on poultry (mosquitoes, midges, gnats, stable flies), only a few are generally of importance due to transmit a number of parasites (Ruff, 1999).

A variety of chemical are used for the control of ectoparasites in poultry. Mullens et al. (2009) reported that early treatment was more effective at controlling fowl mite (*O. sylviarum*) infestation; 85 % of light infestations were eliminated by a pesticide spray (Ravap), versus 24 % of heavy infestations. It is concluded that two administrations of phoxim 50 % (ByeMite®) within a 7-day interval are highly effective against *D.gallinae* infestations in a stocked poultry house (Meyer-Kühling et al., 2007). The costs for control methods and treatment are showing the tremendous economic impact of *D. gallinae* on poultry meat and egg industries (Sparagano et al., 2009). In the Nether-lands, Dutch poultry farmers estimated the costs for preventive and control measures to be €0.14 per hen per round and damage due to RPM because of higher feed intake, higher mortality, and lower egg quality were estimated as €0.29 per hen per round (Emous et al., 2005). Application of parasite control measures must thus takes priority attention

in conjunction with good hygienic practices including separete based on age groups, contruct houses having no craks and crevices is paramount important (Mekuria and Gezahegn, 2010).

#### Conclusion

In the light of this review it is concluded that good management, proper sanitation, good hygiene, use of specific chemicals in the approved manner may most effective in controlling ectoparasites. Further research is to be conducted in to the economic impact of ectoparasites in poultry production.

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# P<sup>29</sup> The Impacts of National and International Standards on Organic Broiler Production in Developing Countries: Turkey Case

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#### Abstract

The characteristics of the organic chicken production process pretty differ than from the conventional. Chicken is deemed organic when it has been produced in accordance with a certain standards. The relationship between organic market and national standards became more important in last decade. For example most organic standards recommend the use of suitable breeds for an organic production whereas EU organic livestock regulation requires breeds for meat production to be of a strain known to be slow growing. There are also minimum slaughter ages for organically kept poultry, this is 81 days for fattening chickens in EU standards and 63 days in BIO-SUISSE. Housing and feeding are important criterias in fattening poultry breeding, but they vary in existing organic standards. All organic standards state that ideally 100% of the diet should be organic, but give some allowance for some non-organic components of the diet under the current situation. Health issues in organic broiler production are controversial. EU and IFOAM regulations permit to use antibiotics whereas USDA NOP prohibits.

As shown there are many differences among standards. Establishing an organic poultry market and improving requires a careful and gradual approach. The aim of this article is to give knowledge about certain requirements for the organic broiler production in different standards and highlights the some problems on organic broiler production.

Keywords: Organic broiler production, organic standards, genotype, housing, feeding, health.

### Introduction

The relationship between organic market and national standards became more important in last decade. Although Turkey is one of the largest poultry producers in the world, share of the organic egg and chicken market is less than %1 yet. The first national legislation on organic animal production published in 2005 and it has been improved according to the EU standards and came into force in 2010 finally. The term organic is clearly defined in EU law and standards have been developed in Turkey in compliance with that EU law. The Turkish Standards implement Regulation (EC) 1804/99, including several more stringent requirements.

The genetic material needed for commercial poultry production is provided from the international breeding companies in Turkey. Hence, only one breeding government research station is performed the genetic selection for egg production with national breeds. The grandparent generation of layers and broilers are imported to Turkey from the international breeding companies. Broilers have a similar production chain as for layers. Turkey does not have a national stock of the grandparent generation for broiler production.

The characteristics of the organic chicken production process pretty differ than from the



conventional. The label "organic chicken" cannot be used loosely. Chicken is deemed organic when it has been produced in accordance with a certain standards. Organic poultry producers must establish preventative health and management practices. Most organic standards recommends the use of suitable breeds for an organic production. Management involves monitoring poultry health; ensuring that the poultry house is maintained with appropriate brooding, rearing, growing conditions; and ensuring that recommended vaccinations are given and appropriate feeding programs are used. On the other hand, for the animal to be considered organic, it would be necessary to utilize techniques that promote the optimization of natural and socioeconomic resources, reducing the dependence on non-renewable energies. The less prepared feed, the more rustic lineages and the lack of antibiotics lead to slower growth of the animal and higher mortality index, reducing the productivity and profitability of organic production.

The aim of this article is to give knowledge about certain requirements for the organic broiler production in different standards and highlights the some problems on organic broiler production. Details relating to standards, derogations, certification bodies, breed choice and suitability, house design and stocking density, outside runs and pasture management, feeding issues, manure usage, marketing and economics need more investigation. Some derogations can be allowed for the developing countries to improve their national organic poultry markets as well as Turkey.

#### Stock and Management

There are differences between breeds/hybrids regarding behavior, feed efficiency, health and production. The literature show that slow or moderate growing genotypes seems to be more suitable for organic production. Most organic standards recommend the use of suitable breeds for an organic production. The EU organic livestock regulation requires breeds for meat production to be of a strain known to be slow growing (1). But the slower growing animals have a less efficient production compared to the very fast growing commercial broilers. Indigenous poultry can cope better with the harsh conditions often prevailing in developing countries, and good management will improve their performance.

Poultry for meat production can only be purchased at one day old and have to be managed according to organic standards for their whole lifetime. Ideally these would be organically reared, but there are derogations until organically reared animals are available in adequate numbers which allow conventionally reared stocks subject to certain limits. These problem is not solved for many countries and because of the lack of an organic breeder, solution seems to not simple. The NOP does not require the origin of the birds to be organic. In fact, there are currently no certified organic poultry hatcheries in the United States. Nonorganic chicks may be used but must be under organic management after the second day after hatching (2). Although slow growing genotypes were used in organic production in the EU, conventional hybrids may be an alternative in developing countries for their national markets.

There are also minimum slaughter ages for organically kept poultry, this is 81 days for fattening chickens in EU standards. It means a problem in practice for commercial breeds, since they grow too fast for kept until 81 days. BIO SUISSE standards that period is 63 days. In Soil Association organic standards also require that organic must be used if available; under organic management after 3 days, must be organic for at least 10 weeks. While conversion period is 10 weeks in EU Standards, its 56 days in BIO-SUISSE.

# 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

#### Housing

Housing is an important criteria in fattening poultry breeding, but they vary in existing organic standards. Houses should be planned <u>appropriate</u> for organic production. Animal welfare and health is considered in planning of house. Housing technics effects on welfare. Feather pecking is the indicator of poor welfare quality and associated with stress (3).

Birds must have access to veranda, fresh air, clean water, balanced ration and area for stratching, and thus presents emphasis to enhance the welfare. Debeaking and beak trimming are usually prohibited practices, however some certifying agencies still permit them. Debeaking if done, more than 5 mm of the upper beak should be removed (4, 5). Veranda allow for animals to exhibit natural behaviors, so veranda increase to welfare (5, 6). However, as flock size increase, veranda useable area decrease (7).

The value of capacity in a house differ from 500 to 4800 chickens in different standards. Soil Association permission is required to keep larger houses. It recommended maximum poultry flock sizes are for a maximum of 500 chickens. Permission is required for larger units, and will depend on good health and welfare levels, good environmental conditions, and sufficient available range with vegetation within suitable distances of the housing. Beyond this, Soil Standards standards restrict the maximum poultry flock size, in any case to 1000 birds (8).

According to EU organic poultry production regulation; for fattening birds in the starter period (0-21 days) a maximum of 20 birds/m<sup>2</sup> and 21 kg/m<sup>2</sup> usable area should apply. At about 3 weeks of age, the average weight may reach 1 kg. In the fattening period (22 to 81 days) the Group recommends that no more than 10 birds/m<sup>2</sup> and 21 kg/m<sup>2</sup> should be permitted (Table 1). Stated in the regulations that inside and outdoor area dimensions are arranged properly. While creating pastures in outdoor area, the limit of 170 kg/N/ha/year should not be exceeded (9).

#### Feed and Feeding

All organic standards state that ideally 100% of the diet should be organic, but give some allowance for some non-organic components of the diet under the current situation. The UKROFS standards allow non-ruminants up to 30% (calculated on a daily basis) from non-organic sources. At least 50% of the diet must be fully organic, and the remaining 20% can come from sources that are in conversion to organic production. The draft EU regulation is proposing stricter rules by reducing the percentage of nonorganic origin for the derogation period to 20% and requiring 60% fully organic (in line with current Soil Association standards following IFOAM accreditation). In addition, the EU draft regulation specifies that holdings should 'normally produce their animal feed themselves' and require special approval to buy in feeds (1). The EU regulation prohibits all animal protein other than milk and milk products. The RSPCA standards also exclude the feeding of animal proteins. The synthetic amino acids is controversial in the organic movement in Europe at the moment. They are permitted under some standards, but either restricted or prohibited under others. IFOAM is intending to revise the standards to prohibit the use of synthetic amino acids in organic rations and the IFOAM EU Group has made similar recommendations to be included in the EU proposal (1).

### Health Care

Sanitary problems, the alternative chickens present some economic difficulties. Health is one of the most important measure of animal welfare. The prevalence of parasitic diseases in organic



production is reported to be higher than conventional breeding (10). Dennett (11) implicates large (2 m) pop-holes and over-exposure to sunlight as a cause of feather pecking and MAFF (12) recommended reduced light intensity to avoid problems.

It is reported that the helminth is an important risk factor in the free rearing systems (13, 14). A lot of treatment with regular anthelmintic may be struggling with helminth parasites, but this chemical regular and preventive use of synthetic drugs is not compatible with the organic regulations and is therefore a need for the development of preventive care and feeding with alternative strategies. Increasing the stocking density was not effect on helminth infection (15) and prevalence of salmonella is not affected from rearing systems (16).

#### Transport

This section requires that stress, injury and suffering be minimized in all handling and transportation of livestock. Transport distances should be kept as short as possible and thus animals should be slaughtered in the region in which they were reared. The driving of animals with electric prods is forbidden. Transport distances shall be kept short, if possible not more than 200 kilometers (8). Animals shall not be treated with synthetic tranquilizers or stimulants prior to or during transport. Each animal or group of animals shall be identifiable at each step in the transport and slaughter process. Slaughterhouse journey times shall not exceed eight hours (8). Although there are clear benefits by using birds selected for organic production, more research is needed that how the commercial breeds could be used in organic production.

#### Discussion

There are many standards in countries and there are differences between them. Regulations in many countries and including European countries are causing some controversy. So the internal and external export production will be considered to establish different standards. Establishing an organic poultry market and improving requires a careful and gradual approach. Organic poultry production in national or international organic standards must be promoted with government support to reach expected market share. In addition, there are limits to the applicability of conclusions drawn from studies performed in other countries, since the prerequisites for organic production may be different in different countries. However, some general rules must be used when discussing the situation in Turkey.

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# 3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

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Criteria	Descriptions	Standards
Stock Management		
Source of	Organic must be used if available or under organic	EU
chicken	management after 3 days.	
	Under organic managements after 2 days.	USDA NOP
	Organic must be used if available; under organic	SOIL ASSOCIATION (UK)
	management after 3 days, must be organic for at least	
	10 weeks.	
Conversion	56 days.	BIO SUISSE
period	10 weeks.	EU
Min.	Min. age: 63 days	BIO SUISSE
slaughter	Min. age: 81 days	EU
age	Similar to EU but depend on slow-fast growing	SOIL ASSOCIATION (UK)
Housing		
Indoor	At least 1/3 area of floor with solid material	EU
floor area	At least 1/2 area of floor with solid material	SOIL ASSOCIATION (UK)
Equipment	Min. feeder space: 2.5 cm/chicken	SOIL ASSOCIATION (UK)
	Min. drinkers: 1 nippel/10 chickens	SOIL ASSOCIATION (UK)
Outdoor	Outdoor access required	USDA NOP
area	At least 1/3 of chickens lives	EU
	At least 2/3 of chickens lives	SOIL ASSOCIATION (UK)
Pasture	Rest pasture for 2 months per year and 1 year in	SOIL ASSOCIATION (UK)
rotation	every 3 years	
Max.	4 m <sup>2</sup> per chicken	EU
outdoor	2,500 chicken per ha	SOIL ASSOCIATION (UK)
density		
Max. flock	4,800 chicken per house	EU
size	500 chicken per house (1000)*	SOIL ASSOCIATION (UK)
Feed and Feeding		
General	%100 organic	USDA NOP
feeding	%100 organic	EU
	Allowed non-organic feed %15	IFOAM 2002
	Careful control required to prevent welfare problems	UKROFS
Animal	No intensive additions	UKROFS
protein	Dairy products, fishmeal	SOIL ASSOCIATION (UK)
	Local certifier to specify	IFOAM 2002
Essential	Prohibited (temporary exception for methionine)	USDA NOP
amino	Prohibited	EU
acids	Prohibited	SOIL ASSOCIATION (UK)
Health Care		
Antibiotics	Not permitted.	USDA NOP
	Permitted as last resort.	EU
	Permitted as last resort.	IFOAM 2002
Transport		
Journey	Max. 10 hours	UKROFS
time	Max. 8 hours	SOIL ASSOCIATION (UK)

Table 1. Certain international standards on organic broiler production.

# P<sup>30</sup> Estimation of Optimum Slaughter Age in Broiler Chicks

### Hasan Çiçek, Murat Tandoğan

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#### Abstract

In this study, optimum slaughter age of the commercial broilers was estimated in terms of maximum net income. Study materials are averages target performance (live weight gain and feed consumption) of Cobb500 and Ross308 commercial hybrids, prices of feed, chick and broiler meat. The optimum slaughter age was estimated by a mathematical function ( $y = f(x) = a + bx - cx^2$ ) that reflects the quadratic (parabolic) relationship. Accordingly, the optimum slaughter age was calculated as 5,62 weeks (39,34 days). As a result, it can be said that genetic improvement affects positively to the correlation between the live weight gain and feed consumption of broilers. However, prices of feed, chick and broiler meat have a more decisive role on slaughter age.

Key words: Cobb500, Ross308, slaughter age, mathematical function



# P<sup>31</sup> High Hydrostatic Pressure; Innovations and Oppurtunities in Poultry Meat Industry for Inactivating Microorganisms

### Burak Bilen, Ahmet Yaman, <u>Gülsün Akdemir Evrendilek</u>

Abant İzzet Baysal University, Faculty of Engineering and Architecture , Bolu, Turkey

#### Abstract

In parallel to increasing demand to poultry products, it has been seen that there is a increase in both the production volume and the product variability. Reduction in the amount of or completely eliminating the food additives to increase shelf life and provide microbial safety of poultry and poultry products are desired from the consumers as well as the producers because they would like produce foods more fresh or fresh like without or with minimal amount of food additives. This fact requires new methods and processes and studies to search alternatives are accelerating in recent years.

Even though applications and/or processes are limited for poultry and poultry products, especially nonthermal technologies come forward to process these products. High hydrostatic pressure (HPP) by inactivating microorganisms and enzymes causing spoilage, provides fresh-like products with increased shelf life of different products. Process conducted at closer to room temperature between 350-650 MPa pressure. HPP is applied for reduction of the marination time, shelf life extension of poultry products, turkey breast and sausage from poultry meat, and depending on the applied pressure and processing time amount of microbial inactivation and shelf life extension were obtained in different products with longer shelf life of HPP processed products. However, more studies are needed to evaluate the potential of HPP for processing of meat and meat products.

# P<sup>32</sup> Effects of Different Supplemental Bentonite on Performance and Levels of some Minerals in Blood Serum and Tissues in Broiler Rations

### Hakan Üstüner<sup>1</sup>, Seher Küçükersan<sup>2</sup>

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#### Abstract

The present study was initiated to determine the effects of supplemental bentonite in diets having on growth performance, feed intake, feed conversion ratio, carcass yield, abdominal fat, some organ weights and the levels of calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn), and P in blood serum, liver and bone tissue in broilers. A total of 288 one-day old broiler chicks (all males) were randomized into 4 experimental groups each containing 72 birds and each group was divided into 4 replicate groups. Bentonite was not added to the diet of the control group where the experimantal diets include 0.5, 1.0 and 2.0% bentonite. Live weight and live weight gain were not significantly different between the groups (p>0.05). The feed intake and feed conversion ratio were significantly higher in the groups having 1.0 and 2.0% bentonite compared with the control group and 0.5% bentonite group (p < 0.01). The pH levels in the small intestine were significantly higher in the 1.0 and 2.0% bentonite supplementation (p<0,01). Dietary bentonite did not significantly effected the carcass yield, weight of abdominal fat, heart, gizzard, liver, proventriculus, bursa Fabricus and spleen (p>0.05). Ca, P and Zn levels in serum were not effected (p>0.05) by bentonite supplementation. Cu serum level were decreased by the increasing bentonite supplementation (p<0.01). Ca, P, Cu and Zn levels in liver and bone tissues were not effected (p>0.05) by bentonite supplementation. Bentonite does not effect the broiler performance when added 0.5% to the broiler feed and bentonite supplementation to broiler feeds sould be considered because of its toxin adsorbent capacity and the cost facts.



# P<sup>33</sup> Effects of Selenium and It's Source on Broiler BreederPerformance, Reproduction and Posthatch Chick Quality

### Özge Pamukçu, Necmettin Ceylan

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#### Abstract

Selenium is important in poultry feeding, is a trace element that represents the essential antioxidant properties. Selenoproteins play important role in protection from oxidation damage to cells by binding to the enzyme glutathione peroxidase.

It has been proved that selenium increased fertility and hatchability in breeders by improving sperm quality of cockorels and also could improve the antioksidant status of developing embryo in breeders fed supplemental selenium. After importance of selenium in performance, product quality and reproduction in poultry has been shown by many studies, more concern and works to organic selenium sources has been established because of very low bioavailability of inorganic sources. So many researches has been done on bioavailability of organic and inorganic sources of selenium , and some new information and data on the positive effects of organic selenium source in broiler breeder diets on reproduction, immune system and post-hatch chick quality. This paper will be focused on metabolism, functions and sources of selenium and it's effects on especially fertility, hatching and post-hatch chick quality in broiler breeders.

**Key words:** Selenium, organic selenium, broiler breeder nutrition, reproduction, hatchery, chick quality

# P<sup>34</sup> Consumer Views for Animal Welfare on Poultry Meat Consumption: Abant İzzet Baysal University Student Union

### Canan Aslan, Ebru Cankurtaran, Eray Yalçınkaya, Esra Deniz, Özde Beyazateş, <u>Ahmet</u> <u>Yaman</u>

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#### Abstract

Poultry meat consumption habit and its relationship with animal welfare are searched among the students educated in Abant İzzet Baysal University main campus area in this study. The survey was performed with 1000 students during 2013 education year. According the survey results, 522 students stayed in house and 478 stayed in dormitories. Students indicated that 363 of them consume poultry meat once a week, 347 of them consume twice a week, and 251 of them consume three times a week. While 555 students prefer red meat; 445 students prefer poultry meat when they are eating out. A total of 570 students out of 1000 indicated that they have never heard animal welfare, and 260 students indicated they have an average or above the average knowledge about animal welfare. A 269 students think that animal welfare, halal meat, and organic products as not much important as the price of the poultry products.

**Key words:** *Animal welfare, poultry meat, consumption preference* 



# P<sup>35</sup> Effects of Freezing and Frozen Storage on Poultry Meat Quality

### <u>Eda Demirok Soncu</u>, Nuray Kolsarıcı, Buğra Bilki, Feyza Yetimoğulları, Hatice Üçler, Yeliz Bayar, Güliz Haskaraca

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#### Abstract

Freezing and frozen storage is a preservation technique which is used to extend the shelf life of chicken meat for many years. But, previous studies have reported that this technique affects negatively quality characteristics of chicken meat although it is successful to extend the shelf life. From this point of view, the changes in proximate composition, color, lipid fraction and protein structure of chicken breast and chicken drumstick which was freezed and stored at -18°C for 48 hours was investigated in this study. The experiment was repeated twice and results were statistically evaluated by using paired samples t-test in Minitab 15.

Freezing, frozen storage and thawing did not affects proximate composition and  $L^*$ ,  $a^*$  and  $b^*$  color values of chicken breast and chicken drumstick (p>0.05). However, as a result of changes occurred in the protein structure of chicken meat, the decrease in water holding capacity of chicken breast and chicken drumstick was calculated as 3.4% and 2.5%, respectively (p>0.05). In paralel with this decrease, an increase was determined in thawing loss and cooking loss values. During thawing, the loss was found to be 0.89% for chicken breast and 0.48% for chicken drumstick. Cooking loss increased by 20% in freezed and thawed chicken drumstick (p>0.05), but this parameter was calculated by 58% for that of chicken breast (p<0.05). While total protein solubility and sarcoplasmic protein solubility showed a decrease in both breast and drumstick, myofibrillar protein solubility increased (p>0.05). When examining the changes in lipid fraction, a significant increase was observed in free fatty acid level of chicken breast and drumstick (p<0.05). In addition, the increase in TBARS value of chicken breast and drumstick was determined insignificant (p>0.05).

**Key words:** *Chicken breast, chicken drumstick, frozen storage, thawing, water holding capacity, lipid oxidation, protein solubility* 

# P<sup>36</sup> Recent Developments in Amino Acid Nutrition of Broiler Breeders

### H. Ozan Taşkesen

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#### Abstract

Performance parameters of broiler breeders has been obviously improved in last decades.this improvement is mainly based on selection and nutrition studies. There has been a need for new nutrition strategies for broiler breeders due to industrial improvement of production, high costs of feedstuff, nitrogen excretion to nature and other problems. In this regard there have been significant new informations and insights on protein and amino acid nutrition of broiler breeders in last years. Improvement of synthetic amino acid industry and availability of many amino acids with relatively reasonable costs along with new scientific information on amino acid digestibility and usage of digestible amino acids are allowing nutritionists to detect new requirements and form new strategies for broiler breeder nutrition in last decade.

Key Words: Broiler breeder, digestibe amino acids, reproductive performance, egg quality



# P<sup>37</sup> Effects of Beta Mannans as Antinutritional Factor in Broilers and β-Mannanase Enzyme to Alleviate It

#### İsmail Yavaş, Gonca Sınacı, Necmettin Ceylan

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#### Abstract

Enzymes are the feed additives that play a key role in the catalytic metabolisms. The biotechnology industry showed a rapid development with the expansion of the use of enzymes. Being able to use in wide range of enzymes and ease of practice play a major role in this rapid growth. Enzymes in poultry feed are used in order to cash on energy utility, intestinal viscosity, intestinal development, immunity and facilitating the digestion of nutrients. Besides this positive effects enzymes are also contribute to enhance animal performance and health and reduce environmental pollution by means of eliminate antinutritional components. Mannans are located in the structure of hemicelluloses classified within non-starch polysaccharides (NSP). Mannans, the characteristics of heat-resistant polysaccharide, are considered as an anti-nutritional factor due to the endogenous enzyme deficiency in poultry. The use of mannanase enzymes has become more important in recent years in order to eliminate anti-nutritional properties of these enzymes. The impact of mannans on poultry animals and the usage possibilities of mannanase enzyme in poultry feed have been evaluated in this paper.

Keywords: mannan, mannanase enzyme, non-starch polysaccharides.

# P<sup>38</sup> Relationship Between Environment and Poultry Nutrition, and Developments

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#### Abstract

Today modern animal management is becoming popular and animal populations are growig in order to maximize the production per animal. However, animal waste also increases with increasing animal population. Poultry feces is closely related with environment due to its nitrogen, phosphor and heavy metal content. Management of this waste is gaining importance due to the effects of excess nitrogen and phosphor on water sources and effects of zinc and copper on soil. Thus, reducing environmentally harmful minerals in animal excreta is an animal nutrition issue that is gaining importance. In this review, nutrition strategies and methods to reduce these minerals, are examined.

Key words: Poultry feces, environmental pollution, feding



# P<sup>39</sup> Effects of A Phytogenic Feed Additive on Breast Meat Yield and Quality in Broiler

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### **Introduction and Aim**

The profitability of broiler production is largely determined by the possibility of increasing the proportion of prime parts in the carcass, mainly breast meat.

On the other side, consumer acceptance of poultry meat is strongly related to meat quality which can be determined by tenderness, juiciness and taste.

Among other factors, genetics, rearing conditions and especially nutrition have been considered to affect poultry meat yield and quality.

Phytogenic feed additives (PFA) are plant-derived products, used in animal nutrition to improve performance and health (Windisch et al. 2008). Additionally PFA have shown to improve carcass meat safety and quality (Mountzouris et al. 2009)

### **Materials and Methods**

<u>Trial 1:</u>

- 432 Ross 308 male broilers
- 2 treatments
  - T1: Control, Corn/soy based diet
  - T2: As T1 + BIOSTRONG 510 at 150mg/kg
- 6 replicates per treatment with 18 birds per pen
- 4 broilers / pen slaughtered on day 28 for meat yield evaluation

Trial 2:

- 288 Ross 308 male broilers
- 2 treatments
  - T1: Control, Corn/soy based diet
  - T2: As T1 + BIOSTRONG 510 at 150mg/kg
- 6 replicates per treatment with 12 birds per pen
- Day 35, 24 broilers / treatment breast meat samples were taken

Breast meat was grilled for organoleptic evaluation

# **Results and Discussion**

In trial 1 no significant effects on daily weight gain were observed. Feed conversion ratio was slightly improved by 1.7% due to feeding the PFA. Birds fed the PFA had a 2.8% higher breast muscle weight (254 g vs. 261 g). In relation to the final body weight, breast muscle content was 4% higher in birds fed the PFA.

Results are shown in Graphs 1

In trial 2 the organoleptic evaluation showed that the PFA only numerically improved juiciness and taste of the breast meat. Tenderness of the meat was significantly (p<0.05) improved in

broilers fed the PFA

Results of the organoleptic evaluation are shown in Graph 2



### Conclusion

Results indicate that phytogenic feed additives (dietary inclusion 150 mg/kg) can increase breast meat yield per broiler and thereby enhance profitability of broiler production Additionally it is shown that phytogenic feed additives significantly improves tenderness of broiler meat.

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# P<sup>40</sup> Effects of Glyceryl Polyethylene Glycol Ricinoleate on Nutrient Utilization and Performance of Broiler Chickens

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## Abstract

A completely randomized design study with a 2 ' 2 factorial arrangement was conducted to observe the effects of glyceryl polyethylene glycol ricinoleate (GPR) on nutrient utilization and performance of broiler chickens. A total of 400 male broiler chickens were used to determine the influence of GPR (with or without), apparent metabolizable energy (AME) level (according to standards or -0.419 MJ/kg of diet) on performance of broiler chickens, apparent total tract digestibility (ATTD) of crude fat and apparent metabolizable energy (AMEn) on day 14, and crude fat and neutral detergent fiber (NDF) total tract digestibility and AMEn on day 35. All diets were based on wheat-maize-soy bean meal with rapeseed and lard (blend of 50:50) as fat sources. Birds fed diets supplemented with GPR were characterized by higher body weight gain (BWG) and lower feed conversion ratio (FCR) than unsupplemented chickens (p < 0.05). Birds fed diets lower in AMEn (-0.419 MJ/kg) were characterized by lower BWG and higher FCR (p < 0.05). Birds fed diets supplemented with GPR were characterized by higher crude fat total tract digestibility than birds whose feed was not supplemented with an emulsifier (d 14 and 35) (p < 0.05). Moreover, GPR addition improved NDF total tract digestibility (35 d) (p < 0.05). The presented results show that GPR addition improves crude fat digestibility and consequently performance of chickens.

Keywords: emulsifier; broiler chickens; fat digestibility

## Introduction

In broiler chickens diets, animal fats and vegetable oils are usually added to increase their energy concentration. In consequence, the birds' growth performance can be improved. Under normal physiological conditions, the gastrointestinal tract (GIT) of a bird is an aqueous environment. Fatty acids have to aggregate to form micelles to get absorbed as hydrophobic components. Emulsifiers, such as bile salts, naturally mediate this process. According to Uni et al. (1999) and Noy and Sklan (1995), in young birds, digestion of dietary fats is limited due to the reduced secretion capacity of bile salts and lipase until the end of second week of life. It could be assumed that addition of bile salts or exogenous emulsifiers to the broiler diets could positively affect fat emulsification and consequently absorption. According to EU legislation, glyceryl polyethylene glycol ricinoleate (GPR), is an emulsifying agent. Literature information about the use of GPR in nutrition of animals is very limited. Amitava et al. (2010) reported that GPR at 1% dose may improve by 5% live weight, which significantly improved feed conversion efficiency. The aforementioned authors showed that GPR effect on fat utilization was evidenced from improvements in apparent total tract digestibility (ATTD) of fat and overall fat metabolizability. Udomprasert and Rukkwamsuk (2006) found that GPR may improve average daily weight gain

and feed conversion ratio (FCR) of weanling pigs. In abovementioned trials conducted with piglets and chickens, authors used vegetable fats to increase their energy concentration. The objectives of this trial were, therefore, to determine the effects of GPR on growth performance as well as nutrient digestibility and AME level of diets of broiler chickens when fed high-fat

Materials and methods

diets and on deterioration of fat in the diets.

The experiment was conducted with 400 male broiler chickens (Ross 308) (initial individual weight:  $40 \pm 2$  g). Ten replicate pens of 8 birds each were randomly assigned to 4 dietary treatments: two positive control (PC) treatments and two negative control (NC) treatments, -0.419 MJ/kg). Diets were supplemented with or without Bredol<sup>®</sup> (0.04%). All diets in mesh form were provided *ad libitum* from day 1 to 35 and were supplemented with TiO<sub>2</sub>, used to calculate digestibility. All nutrients met or exceeded Aviagen recommendations for broiler chickens (Aviagen, 2009). Apparent total tract digestibility and AMEn value of the diets were calculated relative to the ratio of TiO<sub>2</sub> to the content of the nutrient in question in the feed or excreta.

#### **Results and discussion**

From day 14 to 35, a decrease in AME level of diets by 0.419 MJ/kg resulted in lower BWG  $(\sim 1.5\%)$ , (p < 0.1). Use of GPR improves BWG (~64 g/bird), which was particularly evident in NC diet, resulting in a GPR inclusion 'AME level of interaction (p = 0.0162). Birds fed diets with GPR were characterized by lower FCR. Similar differences were obtained considering the whole trial (0–35 d); furthermore, tendencies (p < 0.1) to increase FCR when -0.419 MJ/kg of diet was used were observed. In the starter period, ATTD of crude fat was increased by GPR inclusion (p < 0.05) and not by AME level of diet (p > 0.05). There was a tendency to improve AME level of diet after GPR inclusion (p < 0.1). Determined AMEn level of experimental diets was lower in the NC diet. The difference between treatments was found to be 0.406/kg (PC vs. NC). The GPR diet supplementation improved (p < 0.5) ATTD of GE only in the NC diets, resulting in a significant GPR inclusion × AME level interaction. Here, GPR inclusion resulted in an increase (p < 0.05) of NDF ATTD, independent of AME level of diet. However, the NC diet was more obviously positively affected than PC as GPR was added, resulting in a significant GPR inclusion × AME level of interaction. Multiple comparisons showed that crude fat digestibility was positively (p < 0.05) affected by GPR addition. Finally, a modest improvement was shown (p > 0.05) in the AME level after GPR addition and significantly higher AME level of PC vs. NC diets (p < 0.05;  $\sim 0.456$  MJ/kg). This resulted in a significant GPR inclusion  $\times$  AME level interaction. Meng et al. (2004) speculated that the secretion of lipase and bile salts when calculated per gram of FI is less dramatic. This indicates that the lipase secretion of young birds may not be as inadequate as expected when their FI is considered. This could explain the lack of response of birds after GPR inclusion (0–14 d). Additionally, fat content in practical broiler starter diets is lower than in diets used in the grower or finisher period. Al-Marzooqi and Leeson (1999) reported that used emulsifier had no effect on performance results alone or in combination with lipase. On the other hand, Amitava et al. (2010) found that exogenous emulsifier improved the performance results of birds during the starter as well as grower periods but Zhang et al. (2011) found that a positive effect of exogenous emulsifier was observed only during the starter period. Zhang et al. (2011) and Amitava et al. (2010) concluded that improvement in performance results after emulsifier addition may relate to improvement in observed crude fat digestibility. Abovementioned authors found that emulsifier addition improved digestibility of fat or selected fatty acids. Throughout



this experiment, the emulsifier improved ileal fat digestibility on day 14 and 35. Consequently, as apparent fat digestibility increases after GPR inclusion, higher AME level of diets on day 14 and GE ileal fat digestibility on day 35 were observed. Our findings agree with the report of Zhang et al. (2011), who showed that emulsifier addition improved AME level of diets. However, the reason behind emulsifier's failure to improve AME level of diet on day 35 is unknown. It could be speculated that older birds were characterized by larger capacity of digestive tract, which may mask the positive effects of emulsifiers. In conclusion, the findings of the current study suggest that the positive effects of GPR on blend of animal fat/oil digestion are substantial even in a practical maize-wheat-based broiler diet with decreased AME level. This finding was supported by the detection of interaction between AMEn and GPR addition for fat digestibility at the total tract level and BWG of birds.

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**Table 1**. Influence of diet energy level (AS - according to standards or -0.419 MJ/kg) and GPR inclusion (+) or not (-) on AME level of diet, apparent total tract crude fat (CF), gross energy (GE), and neutral detergent fiber (NDF) digestibility on day 14 and 35.

		Apparen	t total trac	t digesti	bility		
opp to the test		GE	NDF	CF	CF		
GPR* inclusion	AME	35d	35d	14d	d35	AMEn-14d	AMEn-35d
-	AS#	$0.704^{ab}$	0.066 <sup>bc</sup>	0.777	0.814	12.62	12.89 <sup>a</sup>
-	-0.419 MJ†	0.668 <sup>c</sup>	0.028 <sup>c</sup>	0.771	0.796	12.20	11.97 <sup>b</sup>
+	AS	0.695 <sup>b</sup>	0.124 <sup>ab</sup>	0.822	0.845	12.82	12.61 <sup>a</sup>
+	-0.419 MJ	$0.722^{a}$	$0.182^{a}$	0.814	0.846	12.44	12.62 <sup>a</sup>
Model P		< 0.001	< 0.001	0.081	< 0.001	0.011	< 0.001
SEM‡		0.044	0.138	0.059	0.041	0.494	0.077
		Maine e <u>f</u>	fects				
-		0.686	4.7	0.774	80.5	12.41	12.43
+		0.701	15.3	0.818	84.6	12.63	12.61
	AS	0.7	0.095	0.800	0.829	12.72	12.75
	-0.419 MJ	0.695	0.105	0.793	0.821	12.32	12.29
GPR inclusion		0.002	< 0.001	0.011	< 0.001	0.092	0.151
AME		0.504	0.667	0.667	0.128	0.003	< 0.001
		Interacti	on terms				
GPR*AME		< 0.001	0.036	0.958	0.750	0.878	< 0.001

Notes: \* glyceryl polyethylene glycol ricinoleate;

#,† see Materials and methods for information;

‡standard error of the mean;

 $^{abc}$  — means in the same column without a common letter are significantly different at p < 0.05

Table 1. Bird performance (BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio) fed according to standards or -0.419 MJ diets,

supplemented (+)	or not (-) with GPR	[n = 10], [kg	/bird].							
		0–14 d			14–35 d			0–35 d		
GPR* inclusion	AME level	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR
	According to standards#	0.412	0.515	1.254	1.627 <sup>a</sup>	2.591	1.59	$2.04^{a}$	3.105	1.52
	-0.419 MJ	0.386	0.493	1.284	1.566 <sup>b</sup>	2.554	1.63	$1.95^{\mathrm{b}}$	3.047	1.56
+	According to standards	0.388	0.480	1.242	1.656 <sup>a</sup>	2.567	1.55	$2.04^{a}$	3.047	1.49
+	-0.419 MJ	0.390	0.500	1.28	$1.668^{a}$	2.592	1.55	$2.06^{a}$	3.092	1.50
Model P		0.391	0.412	0.555	<0.001	0.612	0.009	<0.001	0.480	0.003
SEM‡ Maine effects		0.006	0.007	0.013	0.009	0.012	0.010	0.011	0.017	0.007
+		0.388	0.490	1.26	1.662	2.579	1.55	2.050	3.069	1.50
		0.399	0.504	1.27	1.598	2.573	1.61	1.997	3.078	1.54
	According to standards	0.400	0.498	1.25	1.641	2.579	1.573	2.041	3.077	1.51
	-0.419 MJ	0.388	0.497	1.28	1.617	2.573	1.593	2.005	3.069	1.53
GPR inclusion		0.384	0.346	0.866	<0.001	0.804	0.002	0.005	0.805	0.001
AME level		0.329	0.934	0.163	0.098	0.789	0.248	0.044	0.821	0.067
Interaction terms										
$\text{GPR}\times\text{AME}$		0.256	0.163	0.798	0.016	0.200	0.318	0.007	0.129	0.339
Notes: * glyceryl #,† see Materials : ‡standard error of <sup>abc</sup> — means in the	polyethylene glycol and methods for info the mean; same column witho	ricinoleate; ormation; out a common	letter are signif	icantly differen	nt at <i>p</i> <0.5					



# P<sup>41</sup> Effect of Dietary Supplementation of Thyme Oil and Vitamin Combinations on PerformanceTraits in Heat Stressed Broilers\*

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#### Abstract

The aim of this study was to investigate the effect of dietary supplementation of thyme essential oil (TEO) and different vitamin combinations (vitamin A, C and E) on performance of heat stressed broilers. A total of 300 male chicks (Ross-308), 21 day old, were divided into 6 groups with 5 replicates. The chicks were exposed to heat stress for 9 h in a day (HS, 34 °C ambient temperature from 08<sup>00</sup> to 17<sup>00</sup>) then thermo-neutral condition for 15 hour in a day (TN, 22 °C ambient temperature from 1700 to 0800) during 21 to 41 d of age. The treatment groups were as follows: 1: thermo-neutral positive control (TNC, 24 h, 22 °C), 2: heat stressed group (negative control, HS, no supplementation), 3: HS and TEO (300 mg/kg), 4: HS and TEO (300 mg/kg)+vit-C (250 mg/kg), 5: HS and TEO+vit-E (250 mg/kg) and 6: HS and TEO (300 mg/kg)+vit-A (15000 IU/kg) supplementation. The body weight (BW), daily gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined at 31<sup>st</sup> and 41<sup>st</sup> d of age. The differences were not significant in BW of groups at 31<sup>st</sup> day and FI at 21 to 31 d of age and BWG of group at 31 to 41 days of age and FCR in whole periods. However, HS caused a decrease in BW at 41th day total BWG and FI (21 to 41 d) of chickens compared to TN positive control group (p < 0.05). The BWG of the TNC and HS group with TEO+vit C, TEO+vit E supplemented groups were not significant at 21 to 31 d and FI in TEO+vit E supplemented group at whole period. In conclusion, heat stress decreased BW and FI traits and dietary TEO+vit C and TEO+vit E supplementation may support BWG and TEO+vit E may contribute FI in heat stressed broilers.

Key Words: broiler, heat stress, thyme oil, performance, vitamin

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# P<sup>42</sup> Investigation of Proteolytic and Lipolytic Spoilage Causing Bacteria for Selling Chicken Meat in Istanbul, Turkey

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#### Abstract

Chicken meat is among the most popular products all over the world including Turkey. Moreover, Turkey is the 9<sup>th</sup> largest poultry producer country in the world. The aim of the study was the isolation of bacteria having strong proteolytic and lipolytic activity, causing spoilage and growing under psychrophilic conditions in chicken meats sold in local bazaar and markets in Istanbul using conventional methods. The strains were identified at the species level by molecular techniques (PCR and DNA sequencing) and the prevalence of bacteria causing spoilage in summer and winter seasons were determined. In this research, a total of 54 samples (22 samples from winter and 32 samples from summer seasons) of chicken meat were collected. In the context of isolation with conventional methods, chicken meat samples were incubated with Standard Method agar and Skim Milk agar for proteolytic bacteria and Lecithin-Lipase agar, Tributyrin agar and Sierra Medium agar for lipolytic bacteria at +4°C for 7 days. After detection of proteolytic and lipolytic bacterial colonies, the isolates were analyzed by biochemical tests such as Gram staining, catalase and oxidase tests. A total of 240 aerobic proteolytic and lipolytic bacteria were isolated 182 strains in winter season and 58 strains in summer season from chicken meat samples. Genomic DNA extraction was performed only strong (+++ and ++++) lipase and protease producing bacteria (total 87; 26 from winter and 61 from summer seasons) using commercial kit with spin colon technology and 16S rRNA gene region was amplified by PCR using gene specific primers. The obtained PCR products (approximately 1500 bp) were sequenced using the same primers. DNA sequencing analysis and comparison with GenBank revealed that 61/87 of strong proteolytic and lipolytic strains in winter season, were identified as predominantly Pseudomonas spp. (17 strains Pseudomonas fluorescens, 17 strains P. fragi, and 9 strains *Pseudomonas* spp.) strains. On the other hand, 26/87 of the strains isolated in summer season were identified as mostly *P.fragi* (n:14), *P. fluorescens* (n:5) and *Acinetobacter* spp (n:4). by comparing obtained DNA sequences from GenBank. According to this study, characterization of spoilage bacteria were determined by molecular biological methods in cold storage (+4 ° C) chicken meat and to extend of the shelf life of these raw chicken meats, it would be appropriate selected specific inactivation methods for identified spoilage microflora, which was identified PCR based methods, was evaluated.

# P<sup>43</sup> Assessment of Poultry Sector in Turkey in Terms of Waste Generation and Waste Valorization

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#### Abstract

Increase in per capita consumption of poultry meat, poultry meat production and exports are indicators of development of the poultry sector as well as being indicators of the increased waste and the problems due to the waste. In recent years, as in other sectors, in poultry sector, an effective waste management is one of the most important activities. Hence, determining waste types and resources that might occur on industrial basis is of great significance. In this context, waste types are examined in three-phases including solid, liquid and gaseous, and waste sources in two parts including farming and processing. This study focused on types and sources of wastes in the poultry industry, and also assessment of poultry sector in Turkey in terms of waste generation and waste valorization.

Keywords: Waste types, waste sources, poultry sector, waste management



# P<sup>44</sup> The First Bivalent *Salmonella* Live Vaccine for Chicken, Turkeys and Ducks

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Food-borne infections with the *Salmonella* (*S.*) serovars *S*. Enteritidis and *S*. Typhimurium are a serious public health concern worldwide. After more than a decade of combating *Salmonella* infections, these organisms still represent an important cause of human disease. Vaccination plays an important role in the overall biosecurity system on chicken farms to prevent *Salmonella* infections. Live attenuated vaccines derived from *S*. Enteritidis and *S*. Typhimurium are widely used and their efficacy, ease of use and outstanding safety under field conditions has been proven. Recently, a new bivalent live vaccine consisting of live attenuated *S*. Enteritidis and *S*. Typhimurium strains obtained marketing authorization in Europe for use in chickens, ducks and turkeys (AviPro® Salmonella Duo).

#### MDM and strain identification

AviPro<sup>®</sup> Salmonella vaccine strains are spontaneous stable mutants selected phenotypically under special culture conditions for reduced growth and prolonged generation time (MDM: metabolic drift mutants, fig. 1). The vaccines are attenuated and rapidly eliminated in the environment. They also carry three independent markers allowing the differentiation between field and vaccine strains by antimicrobial resistance testing. Standard diagnostic methods as well as commercial MIC-microtitre plates (AviPro<sup>®</sup> Plate) are available for diagnostic facilities allowing the fast and reliable differentiation between field and vaccine isolates.

#### Vaccine safety and efficacy

Safety and duration of shedding of the vaccine were proven for chickens, ducks and turkeys (data on file). Standard efficacy tests as described in the Pharm. Europ. were successfully conducted to prove vaccine efficacy after field infections with both *S*. Enteritidis and *S*. Typhimurium. In the challenge experiments, vaccinated chicken showed consistently reduced organ invasion and reduced ceacal colonisation of the challenge strains as compared to non-vaccinated controls (example: fig. 2). Turkeys showed reduced colonization of internal organs with the challenge strains after immunization with AviPro® Salmonella Duo compared to the non-vaccinated control group (example: fig. 3).

Due to the recent emergence of *Salmonella* Typhimurium monophasic strains, an experiment was conducted to confirm the efficacy of AviPro<sup>®</sup> Salmonella Duo by challenge with a recent virulent isolate (fig. 4). An animal model was established to show the efficacy of AviPro<sup>®</sup> Salmonella Duo against the spread of *Salmonella* Enteritidis into eggs. This route of contamination is the most serious public health risk connected to table eggs. The low frequency of this event and thus contaminated eggs makes it difficult to simulate the shedding in a statistically sound way under experimental conditions. This effect could be compensated by using an intravenous high-dosage challenge model which lead to frequent shedding of the challenge *Salmonella* into the laid eggs in the non-vaccinated control group (**Fig. 5**).

3rd INTERNATIONAL POULTRY MEAT CONGRESS BEYAZ ET KONGRESI

Fig. 1: Mutant with retarded growth (MDM-candidate); Photo: Prof. Linde, University of Leipzig



**Fig. 2:** Isolation of *Salmonella* Enteriditis 7 days post challenge (in log). The chickens were 68 weeks old at the time of challenge. The treatment groups were vaccinated at day-old, 6 weeks and 16 weeks of life.



#### Summary and conclusion

Live attenuated vaccines derived from *S*. Enteritidis and *S*. Typhimurium are widely used and have proven their efficacy, ease of use and safety under field conditions. Live vaccines produce better protection than killed vaccines, which have been tested with varying results and only stimulate antibody production.

Killed vaccines can present only those antigens that were induced under the conditions of the fermentation process. Their protective efficacy is additionally restricted by their low immunogenicity in unprimed hosts and the fact that they do not induce cytotoxic T cells. Furthermore killed vaccines do not elicit secretory IgA responses, which play an important role in protecting mucosal surfaces. For this reason, live vaccines should always be used in



Salmonella control, either alone or in combination with inactivated vaccines.

Recently, a new bivalent live vaccine consisting of live attenuated *S*. Enteritidis and *S*. Typhimurium strains obtained marketing authorization in Europe for use in chickens, ducks and turkeys (AviPro® Salmonella Duo). The product consists of co-fermented live attenuated strains of *Salmonella* Typhimurium and *Salmonella* Enteritidis. Now for the first time a bivalent *Salmonella* live vaccine for use in chicken, ducks and turkeys which provides homologous protection against *S*. Enteritidis and *S*. Typhimurium and protection against the monophasic *S*. Typhimurium variant is registered. In chicken, the active immunization with AviPro® Salmonella Duo effectively reduces the fecal shedding and the colonization of internal organs with *S*. Enteritidis and *S*. Typhimurium field strains and the S. Enteritidis contamination of eggs. In ducks it effectively reduces the colonization of internal organs with *S*. Typhimurium. In turkeys the active immunization with AviPro® Salmonella Duo effectively reduces the colonization of internal organs with *S*. Enteritidis and *S*. Typhimurium field strains and the S. Enteritidis contamination of eggs. In ducks it effectively reduces the colonization of internal organs with *S*. Typhimurium. In turkeys the active immunization with AviPro® Salmonella Duo effectively reduces the colonization of internal organs with *S*. Enteritidis and *S*. Typhimurium field strains.









#### 3rd INTERNATIONAL POULTRY MEAT CONGRESS 3. ULUSLARARASI BEYAZ ET KONGRESI

**Fig. 5**: Isolation of *Salmonella* Enteritidis from egg pools (6 eggs/ pool) collected for 3 weeks post i.v. challenge with 5 x10 exp. 7 cfu at week of live 24. Birds were vaccinated at day-old, week 6 and week 16.





## P<sup>45</sup> Nutrigenomics; an Emerging Science in Poultry Production

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#### Abstract

In recent years, parallel to the developments in molecular genetics, not only better definition of feedstuffs but also with increasing awareness of nutrition, nutrigenomics that has become a much debated and interest. Understanding of the relationship between the genetic and nutrition, because of feeds and animal nutrition are the highest cost in livestock sector, nutrigenomic has brought new approach the creation of feeding program. Advances in genomic, proteomic, transcriptomic, and bioinformatic technology have provided a better understanding of the mechanisms underlying these relationships in the nutrition of poultry. By means of these technologies, it can lead to future balanced ration recommendations to promote a healthier animal product and reduce the risks of disease. In this review, it was aimed to give information about nutrigenomic technologies and the relationship between these technologies and poultry animal nutrition.

Keywords : Nutrigenomics, genomic, proteomic, metabolomic, bioinformatic

# P<sup>46</sup> Does Phytase Mean just Ca and P in Broiler Nutrition ?

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#### Abstract

There are some anti-nutritional factors which limit nutrient utilization in animal feeds. Phytic acid that is one of the anti-nutritional factors binds 2/3 of plant origin P. Not only P phytic acid able to bind other minerals like Ca, Mg, Zn, Cu, Fe, K etc. and amino acids and this complex is called phytate. In poultry nutrition phytate is an obstacle to nutrient utilization because phytate able to interfere with digestion and absorption of nutrients in gastrointestinal tract. Due to these reasons phytate degrading enzymes which are called phytase are used widely in broiler nutrition. Phytases break down phytate complex and make available nutrients to digestion and absorption. By this mechanism broilers consume less feed for body weight gain and achieve higher body weights. Recently there are some researches about adding phytase in boiler diets improve bone mineralization, intestinal morphology and antioxidant capacity. Phytase will continue to be major feed additives in broiler nutrition due to minimize environmental pollution and maximize profit.

Key words: phytate, nutrient utilization, phytase



# P<sup>47</sup> Bio-Efficacy of Feed Proteases in Poultry and Their Interaction with Other Feed Enzymes

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#### Summary

The most accepted value of the inclusion of exogenous proteases in poultry diets is the improvement of protein digestibility from dietary ingredients. However, effects of exogenous proteases on animal performance do not necessarily reflect the increment in protein digestibility from proteases in vitro and a variety of factors determine the bio-efficacy of the application of proteases in chickens. An accurate prediction of amino acid digestibility improvements in response to dietary enzymes is important to maximise the animal performance response and ensure that the cost of including the enzyme is justified. Overestimation of the digestibility effect of protease on individual essential amino acids relative to other amino acids may limit the benefits of increments on the absorption of dietary protein for animal growth. A lack of understanding of what effects proteases alone or in combination have on the digestibility of individual amino acids may further imbalance the amino acid profile provided by the diet. It has been recently demonstrated that improvements in the digestibility of amino acids following exogenous protease application in broiler diets can be accurately predicted as a linear function of undigested amino acids in the diet. The contribution of specific proteases in combination with carbohydrase enzymes in terms of protein digestibility has been confirmed. Nonetheless, effects of protease on the digestibility of other nutrients such as fat and fibre, may play a role in determining the *in vivo* response to protease in chickens. Factors like the age of the birds, the gut health status of the animals, and the profile of gut microbial populations appear to affect the response to proteases in animal performance and require further study.

#### Introduction

The use of exogenous proteases in poultry feed has become more prevalent in recent years, following the broader commercial acceptance of other feed enzymes like phytases and xylanases, and increased pressure on the cost of proteinaceous ingredients. Most current commercial proteases for animal feed are alkaline proteases of bacterial origin. Feed cost is reduced with the inclusion of proteases through a reduction of crude protein and first limiting amino acids supplied by dietary ingredients. Nonetheless, the effects of exogenous proteases on animal performance do not necessarily reflect the *in vitro* digestibility of protein from ingredients, but are influenced by a variety of factors that affect their bio-efficacy. For example, serine proteases in broiler chickens do not appear to show a linear dose response on protein digestibility of complete feeds, but an optimum is present (Arguelles-Ramos et al., 2010), after which marginal reductions with increasing protease doses are evident. This suggests that a balance between the hydrolysis of dietary protein and undefined physiological interactions in the intestine may limit further improvements in protein retention due to protease activity. Effects of exogenous proteases are not only confined to the digestion of protein, but extend to the digestion of other nutrients such as fat and starch. Furthermore, the application of proteases cannot be considered in isolation because it often occurs in combination with other feed enzymes, whose mechanisms and subsequent effects on digested nutrients do not appear to be totally independent to those of proteases.

This paper describes factors that influence the bio-efficacy and application of proteases in broiler diets with emphasis on nutrient digestibility, the interaction of proteases with other dietary enzymes, and nutritional effects of exogenous proteases beside protein digestion.

#### Effects of Proteases on Amino Acid Digestibility

A correct estimation of an amino acid matrix to be assigned to exogenous proteases during the formulation of diets is essential to capturing their value in terms of animal performance. That is because an overestimation of the protease effect on the digestibility of essential amino acids, in particular, would create limits to the efficient utilisation of potential improvements on crude protein digestibility. A common mistake in the application of proteases is to assume that a set amount of improvement of protein digestibility, e.g. 3%, can be extrapolated for all amino acids, to calculate an amino acid matrix. In reality, individual dietary amino acids differ widely in their digestibility across different ingredients and diet types. The effect of protease will always be limited by the amount of undigested amino acids present in the small intestine in the absence of the additive. In a recent review, Cowieson (2010) concluded that the inherent digestibility of nutrients in poultry diets prior to enzyme addition is a good indicator of the magnitude of enzyme response.

Romero et al. (2009) conducted a series of studies to determine the relationship between the amount of ileal undigested amino acids and the effects of protease on the ileal digestibility of each amino acid. Four different 21-day digestibility trials were conducted. Each study evaluated the energy and amino acid digestibility of broilers fed corn-soy diets supplemented with a multi-enzyme complex containing xylanase from *T. reesei*, amylase from *B. amyloliquefaciens*, and protease from *B. subtilis* (Avizyme 1502; Danisco Animal Nutrition, DuPont Industrial Biosciences), compared to one containing only xylanase from *T. reesei* and amylase from *B. amyloliquefaciens*, and an un-supplemented control treatment. Diets were corn-soybean meal based in two of the trials, and additionally contained 7-10% corn DDGS in the other two trials.



Figure 1. Percentage change of ileal digestibility of nitrogen and amino acids on a control



diet with addition of two different enzyme combinations of carbohydrases with (XAP) or without (XA) a serine protease in broiler chickens.

On average across all four studies, the addition of the xylanase/amylase combination to the un-supplemented control diet increased ileal digestible energy by 78 kcal/kg, whereas the xylanase/amylase/protease combination increased it by 100 kcal/kg. Most notably, the xylanase/amylase/protease treatment resulted in significantly higher digestibility of nitrogen and all amino acids with the exception of methionine, whereas xylanase/amylase did not exhibit significant differences in amino acid digestibility for any of the evaluated amino acids when compared to the control diets. The amino acids with the greatest digestibility response to xylanase/amylase/protease were cysteine (+5.4%), theonine (+4.4%), glycine (+3.6%), and valine (+3.3%), whereas the least responsive amino acids were methionine (+1.0%), glutamine (+2.0%), lysine (+2.0%), and arginine (+2.1%; Figure 1).

To further explore the reasons for the divergence in the digestibility response to xylanase/amylase/protease of different amino acids, the response relative to the undigested fractions of each amino acid in the control diets was analysed (Figure 2). Interestingly, the amount of individual undigested amino acids at the ileal level clearly determined the amino acid digestibility response to proteases on top of carbohydrases.



**Figure 2.** Uplift of ileal amino acid digestibility of 21-d broiler chickens relative to the amounts of undigested amino acids at the ileal level, in response to carbohydrases or carbohydrases plus a protease in corn/soy or corn/soy/corn DDGS diets. Each point represents measured values for one amino acid in two trials.

Irrespective of the amino acid, a strong linear relationship between the amount of undigested amino acids and the digestibility response to enzymes was evident for xylanase/amylase/protease ( $R^2$ =0.94 and 0.96). These results suggested that protein hydrolysis catalysed by the exogenous protease was responsible for the improvement of apparent ileal digestibility of amino acids. The effect of protease was non-specific to individual amino acids or diet types in this experiment, but it was mostly dependent on the inherent digestibility of amino acids in the diet.

Therefore, the contribution of protease to the digestibility amino acids that are very well digested will be smaller than that for amino acids that are less well digested. It becomes clear that providing greater amounts of highly digestible synthetic amino acids, such as DL- methionine to a diet, must reduce the potential increment in methionine digestibility from a protease. In contrast, amino acids with high concentration or low digestibility, such as glutamic acid, present higher increments on ileal amino acid digestibility from the protease. A conservative approach when recommending matrix values or down-specifications of limiting amino acids for protease supplementation appears to be preferable. Furthermore, digestibility improvement values produced in *in vitro* systems, or *in vivo* systems assessing the digestibility improvements of single ingredients, may overestimate the response of limiting amino acids if the absorption of these amino acids was not properly modelled.

#### Additive Effects of Protease and Other Dietary Enzymes

Effects of protease on poultry diets do not appear to be completely limited to protein digestion, but can also affect the digestibility of other nutrients. McAllister (1993), for example, reported increased digestion of corn starch with the use of a serine protease in a rumen *in vitro* model, which the author attributed to the disruption on the protein matrix in starch granules. Similarly, protein digestibility can also be affected by the presence of other dietary enzymes. Effects of carbohydrases and phytases on amino acid digestibility have been demonstrated and appear to involve a reduction of endogenous amino acid losses (Cowieson et al., 2008; Rutherfurd et al., 2007). It has also been suggested that phytases reduce the association of phytate and protein in the gizzard and proventriculus, increasing protein solubility (Yu et al., 2012). However, as effects of proteases and other dietary enzymes are all dependent on the amount of undigested amino acids present in the digestive tract, increments in amino acid digestibility from different enzymes cannot be additive. Therefore, the nutrient contribution from protease and other dietary enzymes in practical diets should not be determined in isolation.

Romero et al. (2012) conducted a series of studies to better understand the complex interactions of protease with different dietary ingredients and other enzymes. Two studies with 432 21-day or 288 42-day-old Ross-308 broiler males evaluated changes on the ileal energy contribution of substrates in response to xylanase and amylase without, or with protease in four broiler diets. The studies used a 2 x 2 x 3 factorial arrangement of treatments with two base grains (corn-soybeanmeal; or wheat-soybean-meal diets); two fibrous protein ingredient levels (with, or without 10% corn-DDGS and 5% canola meal); and three enzyme levels. At 12 d or 32 d, three enzyme levels were applied: a negative control (NC); NC with xylanase from T. reesei and amylase from B. licheniformis; or NC with xylanase from T. reesei, amylase from B. licheniformis, and protease from B. subtilis (Axtra XAP; Danisco Animal Nutrition, DuPont Industrial Biosciences). At 21 d or 42 d, birds were euthanised; ileal digesta was collected, pooled per cage, and analysed to determine the apparent digestibility of energy, starch, fat, and protein. The increment of ileal energy digestibility of starch, fat, and protein was calculated as the mean change on the coefficient of apparent ileal digestibility of the enzyme treatment compared to the respective control treatment, and multiplied by the measured nutrient content in the diet and the assumed gross energy content of each substrate (starch=4.2 kcal/g; fat=9.4 kcal/g; protein =5.5 kcal/g). Starch digestibility increased with xylanase/amylase (97.8% at 21 d; 96.6% at 42 d) and xylanase/amylase/protease (97.9% at 21 d; 97.0% at 42 d) compared to the NC (96.3% at 21 d; 93.4% at 42 d) across diets. There were no differences between xylanase/amylase and xylanase/ amylase/protease on ileal starch digestion. Xylanase/amylase (84.4%) and xylanase/amylase/



protease (85.8%) gradually increased protein digestibility (P < 0.05) at 21 d (NC=82.7%); but only xylanase/amylase/protease (85.1%) increased protein digestibility compared to the NC (82.4%) at 42 d. Both xylanase/amylase (83.3%) and xylanase/amylase/protease (84.0%) increased fat digestibility compared to the NC (80.2%) at 21 d. At 42 d, xylanase/amylase (86.6%) increased fat digestibility compared to NC (86.6%); and xylanase/amylase/protease (89.4%) further increased fat digestibility compared to xylanase/amylase.



**Figure 3.** Improvement in ileal digestible energy due to supplemental enzymes (◊) and calculated ileal energy contribution from starch, fat and protein fractions (bars) in broiler chickens at 21 (A) and 42 (B) days of age. CS=corn/soybean meal-based diet; WS=wheat/ soybean meal-based diet; HFI=high-fibre ingredients (corn- DDGS and canola meal); XA=xylanase and amylase; XAP=xylanase, amylase and protease enzymes.

At 21 d (Figure 3), the largest contributor to the increase in apparent ileal digestible energy (AIDE) with xylanase/amylase/protease was the protein fraction, and the protein contribution to digestible energy was greater in wheat than in corn-based diets. For xylanase/amylase, there

was a similar trend with the exception of corn-based diets without high fibre ingredients such as corn-DDGs, where the protein contribution to the effect of the enzyme was relatively low. Evidently, fat digestibility had a more prominent role in the total energy effect in response to enzymes in wheat-based diets, whereas the contribution of fat digestibility in corn-based diets was marginal. The extent whereby increments in starch digestibility contributed to the energy contribution of xylanase/amylase or xylanase/amylase/protease was also different between diets, being greater in wheat- than in corn-based diets. At 42 d (Figure 3), the energy contribution from increments in protein digestibility was still relatively high compared to that of starch and fat. Energy contributions from fat were high in 21 d old chickens, but substantially less at 42 d. Only marginal improvements on the energy contribution from starch and fat due to protease were present at 42 days; however, they represented between 9 and 38 kcal/kg depending on the diet type.

Interestingly, the measured energy improvement from enzymes in corn-based diets closely resembled the sum of the contributions from digested starch, fat, and protein at 42 d. However, in wheat-based diets there was a consistent difference between the measured increase in ileal digestible energy from enzymes and the calculated contributions from starch, fat, and protein. This suggests that the digestibility of other components in the diet (other than fat, starch, and protein) may have contributed to the increased AIDE improvements due to enzymes. Enzyme inclusion may have caused either an increased fermentation of NSPs or the absorption of pentose sugars in the small intestine. The fact that this difference was not present in 21 d, but only in 42 d chickens also suggests interactions with the microbial populations of older birds may have occurred, with a resultant increase in digestion of fibre in the small intestine.

These data demonstrate that, although the main effect of protease was an improvement of protein digestibility, carbohydrases also significantly contributed to protein digestibility at least in some diets and for some growth periods. Similarly, proteases can also contribute to increasing the digestibility of other nutrients and presumably the digestion of NSPs. Other studies have also found overlapping effects of protease and carbohydrases on the digestibility of fat and starch in broiler chickens (Kalmendal and Tauson, 2012). Variability in the digestible nutrient response that is frequently seen with individual enzymes may be ameliorated by an integrated approach to exogenous enzyme utilisation which does not attribute independent additive nutrient contributions to single enzyme activities. It must also be stressed that the interactions of carbohydrases and proteases are likely to be diet- dependent and carbohydrase enzyme effects may differ depending on the nature of the NSPs in different grains and vegetable protein ingredients.

#### Protease Effects on Fibre Digestion In Chickens

Effects of exogenous proteases on the digestion of fibre in *in vitro* rumen systems have been reported (Colombatto and Beauchemin, 2009). However, specific effects of protease on the digestion of fibre in poultry are not well understood. Olukosi and Romero (2012) recently studied the effects of different protease doses, with or without carbohydrases on the nutrient digestibility and the NSP flow of broiler chickens fed corn/soybean-meal diets with the inclusion of corn-DDGS. A total of 336 1-d old broilers received a standard broiler starter diet until day 14 when they were allocated to seven treatments in a randomised complete block design. Each treatment had 8 replicate cages with 6 birds per replicate cage. Diet 1, the control, contained no enzyme, diets 2 and 3 contained protease from *B. subtilis* at graded levels (protease 1; 5000 or



10000 u/kg), diet 4 contained another bacterial protease (protease 2; 10000 u/kg) whereas diets 5, 6 and 7 contained admixture of xylanase from *T. reesei*, amylase from *B. licheniformis*, and protease from *B. subtilis* (Axtra XAP; Danisco Animal Nutrition, DuPont Industrial Biosciences) at graded levels (50%, 100% and 200% the recommended dose for broilers, containing 2500, 5000, or 10000 protease u/kg). The diets were fed for 7 days, excreta samples were collected for the last 3 days of the experiment and ileal digesta samples were collected on the last day of the study.

The total tract flows of arabinose, xylose, galactose, glucose, and glucuronic acid from the NSP fraction (Figure 4) were reduced at the higher doses of protease, and both the intermediate and the high dose of xylanase/amylase/protease. In the specific case of xylose and arabinose, which are the main sugar components of arabinoxylans, the inclusion of xylanase and amylase generally reduced the flow of these sugars compared to protease alone at a comparable dose. These data suggest that fibre degradation may be one of the mechanisms by which proteases increase the digestion of nutrients in chickens. Although the reason of these effects of protease on NSP digestion is not known, studies in ruminant models have suggested that the use of proteases can disrupt cell wall associated proteins, which facilitates microbial colonisation of the substrate (Colombatto and Beauchemin, 2009). However, effects of exogenous proteases on the gut microbial populations of chickens have not been properly studied.



**Figure 4.** Total tract flow of total sugars from the non-starch polysaccharides (NSP) fraction in 21-d-old broilers fed corn/soybean meal diets supplemented with different doses of two bacterial proteases or a combination xylanase, amylase, and protease (XAP).

#### **Other Factors Affecting The Bio-Efficacy of Proteases**

Effects of feed proteases that are not directly related to nutrient digestibility have also been reported in the literature. For instance, Caine et al. (1998) reported a reduction in the level of trypsin inhibitors of soybean meal with the use of a serine protease, which may be present in practice and has not been fully recognised as one of the effects of value for poultry diets. Positive effects of exogenous proteases on the ability of birds to cope with intestinal disease challenges like *Eimeria* infections or necrotic enteritis have also been suggested, although mechanisms are not well understood and the evidence available is not definitive. Peek et al. (2009) found that a

protease from *Bacillus licheniformis* increased the body weight gain of broilers challenged with three *Eimeria* species, and suggested that the mechanism was a reduction in the attachment of parasites to the mucus layer. This was supported by an increase in the thickness of the adherent mucus layer of the intestine due to protease. Yan et al. (2011) suggested that a protease avoided the growth of *Clostridium perfringens* in birds challenged with an *Eimeria* vaccine through improved absorption of protein and a reduction in the protein available for bacterial growth. However, other reports have not found clear effects of dietary protease on birds challenged with *Eimeria* vaccines (Walk et al., 2011). Nonetheless, intestinal health appears to be a factor that affects the animal performance responses in the field with the use of proteases, and require further study.

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