XVIII EUROPEAN SYMPOSIUM ON THE QUALITY OF EGGS AND EGG PRODUCTS AND XXIV EUROPEAN SYMPOSIUM ON THE QUALITY OF POULTRY MEAT
23-26 JUNE 2019 CESME-IZMIR, TURKEY

BOOK OF ABSTRACTS

HOSTED BY THE TURKISH BRANCH OF WORLD'S POULTRY SCIENCE ASSOCIATION
WORLD’S POULTRY SCIENCE ASSOCIATION
TURKISH BRANCH

ABSTRACT BOOK

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XVIII European Symposium on The Quality of Eggs and Egg Products and
XXIV European Symposium on The Quality of Poultry Meat (2019, Çeşme)

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ISBN: 978-975-97654-1-5
Welcome from Chairperson of the
XVIII European Symposium on the Quality of Eggs and Egg Products and XXIV European Symposium on the Quality of Poultry Meat

Prof. Dr. Rüveyde Akbay

On behalf of the Organizing Committee and the WPSA Turkish Branch it gives me a great pleasure to welcome you all to the “XVIII European Symposium on the Quality of Eggs and Egg Products and XXIV European Symposium on the Quality of Poultry Meat”. This symposium will provide a platform for the exchange of new ideas, information on the latest advances and for building up and strengthening professional relationships. The Working Groups No. 4 and 5 are traditionally one of the major groups of the European Federation of WPSA. XVIII European Symposium on the Quality of Eggs and Egg Products and XXIV European Symposium on the Quality of Poultry Meat”, like past similar events will be a gathering where world-renowned scientists will share their knowledge, keep us abreast of the developments in the field and show the way for the future research and practice. I would like to express my sincere gratitude to the esteemed scientists who will make this symposium more meaningful with their presentations and the sponsors who made this symposium possible.

I wish you all an enjoyable and productive time at the symposium and a wonderful stay in Çeşme.

Prof. Dr. Rüveyde Akbay
President of the WPSA Turkish Branch
Head of the Organizing Committee

Welcome from President of the European Federation of the WPSA Branches

Prof. Dr. Estella Prukner-Radovcic

Dear Colleagues and Friends,

On behalf of the European Federation of the World Poultry Science Association, it is my great pleasure to welcome all of you and be with you here in Çeşme at the XVIII European symposium on the quality of eggs and egg products and XXIV European symposium on the quality of poultry meat.

I know that the success of the conference depends ultimately on the many people who have worked in planning and organizing both the scientific program and supporting social arrangements. I want to take this opportunity to thank the Turkish Branch of WPSA for hosting this meeting and I would like to express deep appreciation to all members of the Organizing and Scientific Committees, especially the presidents’ prof. Rüveyde Akbay and prof. Servet Yalçın, and the chairpersons of working groups 4 and 5, for all their hard work for making the Symposia a success.

The organizers have developed an attractive scientific programme with numerous speakers from diverse countries who are well known for their expertise. Especially thanks to the contributions of outstanding invited speakers. Next days will be dedicated to cover a wide spectrum of themes related to the quality of eggs and egg products and the quality of poultry meat and should provide the meeting place for all involved in those areas of poultry science, offering a bridge between academia and industry.

I am sure that you will find this Book of Abstracts a very informative and useful one containing abstracts of all papers and posters to be presented, and will also remind you of the impressive days in Çeşme.

I’d like to thank each of you for attending the Symposium and wish you an enjoyable and pleasant stay in Turkey.

Estella Prukner-Radovcic
President of the European Federation of the WPSA Branches
Welcome from Chairpersons of Working Group 4

Dr. Cristina Alamprese and Joel Gautron

Dear Colleagues and friends,

As the chairpersons of WG4, it’s our pleasure to welcome you to the XVIII European Symposium on the Quality of Eggs and Egg Products. The Symposium is organized by the Turkish Branch of WPSA and, as usual since 1989, it is run in parallel with the XXIV European Symposium on the Quality of Poultry Meat. The two Symposia represent a unique opportunity to share new findings about poultry products and to discuss the actual challenges of this important sector. The egg production has to face many new challenges, such as the increased demand for “natural outdoors or organic” eggs and the longer period of layer production requiring major changes to maintain egg hygiene and quality. We believe that the outstanding scientific program arranged by the Scientific Committee will provide excellent “food for thought” in order to promote research and industrial activities in the field of egg and egg product quality. Similarly, we are sure that the social program developed by the Organizing Committee will provide “food for the soul”, giving us the opportunity to interact, make new friendships and exchange ideas for future projects.

Enjoy the symposia and your time in Turkey!

Cristina Alamprese and Joel Gautron
Chairpersons of the Working Group 4 “Quality of Eggs and Egg Products”

Welcome from Chairperson of Working Group 5

Prof. Dr. Massimiliano Petracci

Dear Friends and Colleagues,

First, let me thank the members of Turkish Branch WPSA and Scientific Organizing Committee for their commitment to the EggMeat Symposium 2019. I would also like to acknowledge the professionalism of the Referee that reviewed the abstracts of this volume.

During the last few years, it has been my distinct honour and privilege to serve as chairperson of WG5 of which the European Symposium on the Quality of Poultry Meat is the main focus. Since 1989, the Symposium is run in parallel with the Symposium on “Quality of Egg and Egg products”, and through the years, EggMeat Symposium has been able to continue providing a platform for discussing the issues, challenges, opportunities and findings in poultry meat and egg quality. The ever-changing scope and rapid development of poultry sector create new problems and questions, resulting in the real needs for sharing brilliant ideas and stimulating good awareness of this important research field. For this reason, I look forward to watching this conference evolve over time and to learning more at this and future EggMeat Symposia.

Please enjoy the scientific program and continue to pave the way for better knowledge on all complex aspects involving poultry meat quality.

Massimiliano Petracci
Chairperson of Working Group 5 “Poultry Meat Quality”
Poultry products quality for meeting future food demands
Roel Mulder

A Miracle: Egg Consumption And Human Health - Literature Review Of 50 Years
Bingir Sönmez

Will vegetarianism, veganism and in vitro meat replace poultry meat?
Michael Grashorn

Novel egg components and human health
Sophie Réhault Godbert

Invited Speakers

Opportunities for genetic improvement in egg quality
Ian C Dunn

Internal egg quality - future challenges
Dietmar Flock, Michael Grashorn

Eggshell contamination and risk for food safety
Juliet R. Roberts

Egg shell quality: Affecting factors and mineralization
A. Rodriguez-Navarro, Spain

Keel bone damage in laying hens
Michael Toscano

Hen nutrition and egg quality
Yves Nys
Oral Presentations

Interest of using imputation for genomic selection in layer chicken
Florian Herry, David Picard Druet, Frédéric Hérau, Amandine Varenne, Thierry Burlot, Pascale Le Roy, Sophie Allais

Guinea fowl eggshell structural organization and particular organic matrix protein patterns to decipher its exceptional mechanical properties
Nathalie Le Roy, Lucie Combes Soia, Aurélien Brionne, Valérie Labas, Alejandro B Rodriguez Navarro, Yves Nys, Joël Gautron

Identification of extracellular vesicles involved in the biomineralization of the hen eggshell
Lilian Stapane, Nathalie Le Roy, Jacky Ezagal, Joël Gautron

Feed efficiency and Egg Quality of Mid Laying ISA Brown Hens
Doreen Onyinye Anene, Yeasmin Akter, Cormac John O’shea

Long-term supplementation 25-hydroxyvitamin D3 on bone growth and development, egg production and egg quality in pullets and laying hens
Chongxiao Chen, Bradley Turner, Gilberto Litta, Todd Applegate, Woo Kyun Kim

Egg white powder as gelling and stabilising agent for double emulsions
Cristina Alamprese, Maria Eletta Moriano

Rheological behaviours of modified liquid egg albumen with lipase enzyme
Hatice Asik, Muhammed Yuceer, Cengiz Caner

Assessment of furosine formation and changes in the quality of eggs treated with hot water during storage
Buşra Erol, Ferruh Erdöðda, Figen Korel

Effects of genotype and age on eggshell cuticle coverage in modern hen strains
Federico Sirri, Paolo Ferrari, Marco Zampiga, Adele Meluzzi, Annachiara Berardinelli

Implications on bioequivalence of HMTBa and dl-methionine
Nizamettin Şenköylü

Influence of feeding faba beans with high and low vicin-content on the egg quality of two local and one commercial chicken breed
Tanja Nolte, Simon Jansen, Steffen Weigend, Daniel Moerlein, Ingrid Halle, Wolfgang Link, HennerSimianer, Ahmad Reza Sharifi

Effect of a novel phytase on P content in egg components and on egg production of laying hens
Roger Davin, Laura Star, Cees Kwakernaak, Dieter Feuerstein

The effect of feed structure on egg quality traits of laying hens reared in conventional and enriched colony cages
Mehmet Bozkurt, Bahattin Koçer, Gökhan Ege, Ahmet Engin Tüzün, Meltem Öztürk

Egg quality traits of Isa brown hens fed fonio (Digitaria iburua)-based diets
Oluwatosin Oluwamoroti Ohotuowo Kennedy, Comfort Ini Ukim, Aloysius Ausaji Ayuk, Leonard N Agwanobi, IsioEkong Isio

The effects of different plant extracts supplemented to layer diets enriched with omega-3 fatty acids on egg efficiency, quality, lipid peroxidation and antioxidant capacity: antioxidant status and egg sensory characteristics
Burcu Aktaş, Hatice Basmacoğlu Malayoğlu

Are omega 3 eggs risky for human health?
A. Yannakopoulos, A. Tserveni-Gousii and E. Yiannakakis

5 Minutes Oral Presentations

The effect of housing system and storage time on cuticle deposition and egg weight of Isa Brown’s eggs
Mohamed Ketta, Eva Tumová, Darina Chodová

Selection for blue eggshell in Country chicken breed
Châhkang Lin, Yench’i Ku, Chihfeng Chen

Physiochemical and functional properties of protease, lipase and phospholipase a2 enzyme-modified liquid egg’s white
Muhammed Yuceer, Cengiz Caner

Effect of ultrasound processing on the physico-functional characteristics of liquid egg yolk
Muhammed Yuceer

Effects of Bacillus subtilis PB6 supplementation on intestinal microflora, litter condition, and some blood parameters of broiler breeder hens under commercial farm condition
Mojtaba Zaghari, Hosna Hajati, Elham Darsi

Effect of Camelina sativa oil or camelina meal in diets for laying hens on chemical composition, cholesterol content and fatty acid profile of egg yolk lipids
Sylwia Anna Orczewska Dudek, Mariusz Pietras

Effects on egg quality of using grape seed oil in laying hen diets enriched in polyunsaturated fatty acids
Raluca Paula Turcu, Margareta Olteana, Tatiana Dumitrapanaite, Mariana Ropota
Chestnut tannins in laying hen feed and their effect on metabolism during the laying peak
Kobe Buyse, Beatrice Wegge, Evelyne Delecize, Geert GP Janssens, Marta Lourenço

Effect of dietary supplementation of hydroxy-selenomethionine on egg albumen quality
Maria Palomar, Antonio Hernández, María Dolores Soler, Michele Demarco, Bea Abad, Carlos García

Effect of different storage conditions on the change of egg quality traits of organic eggs
Musa Sarıca, Umut Sami Yamak, Kadir Erensoy

Effect of dietary blackberry and nut tree leaves on egg quality
Tatiana Dumitra Panaite, Rodica Diana Criste, Arabela Elena Untea, Alexandra Lupu, Mihaela Saracila, Raluca Paula Turcu, Teodor Gavris, Ionelia Taranu

The effect of rosemary and fennel volatile oil on performance and egg quality parameters in layer quail diets
Derya Yesilbag, Ismail Cetin

Orange peel oil supplementation in laying quail diet: Effects on performance and oxidative stability of eggs yolk
Metin Çabuk, Serdar Eratak, Mehmet Bozkurt, Buket Gelir

Calcium pidolate improves egg quality when it is fed to commercial layers from 50 weeks of age
Maureen Bain, Tim Parkin, David Brass, Benjamin Pollet

Poster Presentations

Quality of functional foodstuffs based on the egg mélange
Izabella Lvovna Stefanova, Vladimir Kimovich Mazo, Ludmila Vasilevna Shakhnazarova, Anastasiya Yurevna Klimenkova, Alexsey Shamilovich Kvatvarashvili

Innovative Eggs and Egg Products
Muhammed Yuceer, Cengiz Caner

Effect of Bacillus subtilis DSM29784 on cecal digesta microbiome and links to the concentration of short chain fatty acids and apparent retention of dietary components in Shaver White pullets during grower, developer and laying phases
Damien P Preveraud, Pascal Thiery, Miguel Colilla, Baris Yavuz, Neijat Mohamed, Elijah Kiarie Junko Noguchi

Effect of citric acid addition on physical properties of liquid whole eggs
Nives Marušič Radovčič, Anet Režek Jumbrak, Sven Karlović, Helga Medić

The effects of licorice root powder (Glycyrhriza glabra) on microbiological load of hatching eggs and feces of laying japanese quail
Ayşen Bulancak

Epidemiological evaluation of reproductive traits and economic of feed and hatchability losses in broiler breeders
Nasir Mukhtar, Javid Iqbal, Sohail Hassan Khan, Tanveer Ahmad, Riaz Hussain Pasha

Effects of rearing systems and age on egg yolk composition of Bovans White layer hybrids
Yasin Baykalır, Ulku Gulcihan Simsek, Ökkes Yılmaz

Implementation of “farm to end-point-consumer in 48 hours” by introducing artificial intelligence (AI)-based Layer-Egg-Optimization system
Shau Ping Lin, Lee Tian Chang, Yun Heh Jessica Chen Burger, Yang Han Lee, Chih Yi Layra Yang, Chih Yun Yoyo Yu, Georgios Kalokyris, Kin Hing Frederick Phou, Chih Li Julie Sun, Cheng Chih Hsu, Jia Kun Chen, Wen Chin Yang

The egg based foodstuff for the prevention of the disorders of carbohydrate and lipid metabolism
Izabella Lvovna Stefanova, Elena Vladimirovna Kropacheva, Anastasiya Yurevna Klimenkova, Vladimir Kimovich Mazo

Microbial hazards and risks in industrial Eggs and Meat in Georgia
Kakha Nadiradze

Effects of Bacillus subtilis PB6 supplementation on productive performance, egg quality, and hatchability in broiler breeder hens under commercial farm condition
Mejtaha Zaghari, Elham Darsi, Hosna Hajati

Quality of functional foodstuffs based on the egg mélange
Izabella Lvovna Stefanova, Vladimir Kimovich Mazo, Ludmila Vasilevna Shakhnazarova, Anastasiya Yurevna Klimenkova, Alexsey Shamilovich Kvatvarashvili

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Effect of citric acid addition on physical properties of liquid whole eggs
Nives Marušič Radovčič, Anet Režek Jumbrak, Sven Karlović, Helga Medić
Molecular methods to predict meat quality
C. Berri, S. Benuclercq, E. Pampouille, C. Hennequet-Antier, C. Praud, E. Le Biihan-Duval

Strategies to reduce incidence of myopathic defects
Sacit Bilgili

Risk assessment contaminants in poultry meat
M. Hafez

Impact of increasing incidence of meat quality aberrations on poultry processing
Francesca Soglia, Giulia Baldi, Massimiliano Petracci

Dietary fat by-products and broiler meat quality
Ana Cristina Barroeta, Roser Sala, Alba Tres, Francesc Guardiola

Characterization of single-nucleotide GDF8, WWP1 and PPARC1A polymorphisms genes affecting skeletal muscle growth and energy metabolism in broiler chickens
Cinzia Marchitelli, Michela Contò, David MeoZilio, Sebastiana Failla, Monica Guarino Amato

The relationship between metatarsus (shank) and some body characteristics in broiler pure line and cross genotypes
Kadir Erensoy, Musa Sarıca, Moise Noubandiguim, Umut Samı Yamak, Ali Tekgüler, YeşimBenalÖztekin, Tuğba Karaköse

Longitudinal RNA-seq Analysis of Tissue Development Reveals the Hub Genes that Influencing the Chicken Intramuscular Fat and Abdominal Fat Deposition
Siuyan Xing, Ranran Liu, Richard P. M. A. Crooijmans, Lu Liu, Ole Madsen, Zhou Wu, Huaxtian Cui, Qinghe Li, Maiqing Zheng, Guiping Zhao, Martien A. M. Groenen, Jie Wen

Fatty acid profile in liver of different poultry genetic strains
Alice Cartoni Mancinelli, Alessandra Di Veroli, Gabriele Cruciani, Elisa Cotozzolo, Alessandro Dal Bosco, Cesare Castellini, Simona Mattioli

Effect of post-hatch holding time and feed access time on live performance and carcass characteristics
Serdar Özlü, Ahmet Uçat, Roger Banwell, Okan Elıbul
Effect of chickens breed, lysine depletion and feed form on breast meat quality
Solène Toussaint, Stéphanie Klein, Nicolas Brévault

Alternative protein feeds for a sustainable future: Resulting broiler meat quality
Brianne A. Altmann, Daniel Mörlein

Feeding crude corn oil as by-product of ethanol industry to broiler chicken
Márta Erdélyi, Róbert Kecskés, Zsolt Anscin, Csaba Fernye, Andrea Bócsai, Krisztián Balogh, Miklós Mézes

Potential of probiotic (Bacillus subtilis) and carbohydrase enzymes in improving nutritional value of pea meal in broiler diets
Pawel Konieczka, Jan Jankowski, Krzysztof Kozlowski, Katarzyna Zabek, Marcin Barszew, Stefania Smulikowska

Positive Effect of 25-OH-D3 on broiler meat quality defects
Verane Gigaud, Jean Marc Thoby

Effect of zinc source and level on broiler carcass defects at market age
Cibele Torres, Francisco Fernandez, Tim Horne, Marco Rebollo

Meat proximate composition and fatty acids profile of Muscovy duck fed with partially defatted Black Soldier Fly meal
Marta Gariglio, Sihem Dabbou, Francesco Gai, Sara Bellezza Oddon, Angela Trocín, Antón Pascual Guzmán, Laura Gasco, Achille Schiavone

Evaluation of rosemary extract and ascorbic acid in different phases of emulsion gels on the quality characteristics of model system chicken meat emulsions
Hülya Serpil Kavuşan, Elnaz Sharefi Abadi, Hilal Can, Burcu Sarı, Meltem Serdaroğlu

Effects of using egg shell powder as phosphate replacer on quality properties of chicken patties
Elnaz Sharefi Abadi, Damla Tabak, Meltem Serdaroğlu

Anti-inflammatory capacity of phytogenic compounds to improve the resilience of broilers against intestinal infections
Jan Dirk Van Der Klis, Francisco Dias, Manu De Laet, Andreas Mueller

A novel way to suppress growth of Campylobacter in chickens prior to processing
Tamsyn M Crowley, Ben Wade, Sarah Shigdar, Anthony Keyburn

5 Minutes Oral Presentation

Using a probiotic to ensure food safety
Wouter Van der Veken, Shahram Golzar Adabi, Veerle Hautekiet

Poster Presentations

The comparison of production and meat quality of turkey crossbreds from Old Hungarian and commercial breeds
Rubina Tünde Szabó, Árpád Drobynyák, Mónika Heininger, AgnesZimborán, László Bödi, Károly Kustos, ÁdámCsányi, Mária Kovács Weber

Broiler genetic strain affects breast meat quality, histology, white striping incidence and gene expression
Servet Yalcın, Mustafa Akşit, Guldehen Bilgen, Bulent Helva, Sezen Orzcan, Gamze Turgay Izzetoglu

Effect of lighting program on carcass features and meat quality in broilers
Yasin Baykaltır, Ülkü Gülcihan Şimşek, Gülşah Güngören, Alper Göngören, ÖnderOrlu

Causes of carcass condemnations of broiler chickens at industrial slaughterhouse of Ardabil, Northwest of Iran
Aşid Azizor

The meat production of Yellow Hungarian Chicken breed in different keeping systems
Árpád Drobynyák, Mónika Heininger, Károly Kustos, László Bödi, Rubina Tünde Szabó, Szonja CsengeS kóbár, István Szalay, Ágnes Zimborán, Mária Weber

Specific activity of intestinal enzymes in response to immediate and delayed access to feed and water in broiler chickens
Reza Mirbakh, Shrin Honarbaksh, Seyed Davood Sharifi, Saeed Aminzadeh

Technological Quality of Chicken Nuggets Formulated with Pumpkin Powder as Wheat Flour Replacers
Burcu Öztürk Kerimoğlu, Hülya Serpil Kavuşan, Melih Sarıcalı, Dilara Kişi, Meltem Serdaroğlu

Study of the prevalence of Salmonella in poultry slaughterhouses and cutting plants
Hang Zeng, Koen De Reu, Sarah Gabriel, Lieven De Zutter, Geertru Rascchaert

Effects of dietary organic and inorganic selenium on the quality aspects, nutritional and shelf life of goose meat
Kazem Alirezalu, Zabihollah Nemati, Mohammadreza Hajipour and Maghsoud Besharati

Quality and shelf-life stability of meat and liver from goose fed diets supplemented with vitamin E
Kazem Alirezalu, Zabihollah Nemati, Mohammadreza Hajipour and Maghsoud Besharati

The relationship between air deformation test and meat quality traits of wooly breast fillets
Xiao Sun, Casey M. Owens

Gaping of pectoralis minor muscles: an emerging quality issue for broiler processors?
Francesca Soglia, Andressa Kühnen Silva, Giulia Baldi, Massimiliano Petracci
Examining the objective quality parameters of pigeon meat
Mária Kovács Weber, David Kovács, Rubina Tünde Szabó

Electrical stunning of poultry: influence of animal sex and weight
Marie Bourin, Elisabeth Baeza, Thierry Bourdeau, Cécile Berri, Christophe Souchet, Céleste Le Bourhis, Laure Bignon

Evaluation of lipid and protein metabolism of glycolytic muscles to identify animals able to perform their locomotor behavior
Michela Contò, Cinzia Marchitelli, David Meozilio, Francesco Cenci, Gianluca Renzi, Sebastiana Failla, Monica Guarino Amato

Optimization of the gut health in broilers received yeast (Saccharomyces cerevisae) combined with threonine in diets
Armando Jesús Nilson, María Fernanda Peralta, Raúl Daniel Miazzo

Antioxidant effect of E vitamin in broiler breast meat at different times post slaughter
Armando Jesús Nilson, María Fernanda Peralta, Fernando Mañas, Raúl Daniel Miazzo

Reducing Campylobacter contamination via a probiotic feed supplement
Wouter Van der Veken, Shahram Golzar Adabi, Veerle gHautekiet

Effect of pumpkin seed meal on physicochemical and sensory traits of broiler meat
Ivica Kos, Dalibor Bedekovic, Vjera Zoric, Ivan Vruce, Zlatko Janjec

Growth response in protease induced canola meal based diets with low amino acids levels in meat type broiler under hot environment
Nasir Mukhtar, Laeq Ahmad, Sohail Hassan Khan, Naeem Mehmood Ashraf

The effects of plant mix extract (honokiol-magnolol-sanguinarine) supplementation on performance and biochemical parameters in quails diets
Deniz Belenli, Ismail Çetin

The effects of age and different dietary protein inclusion levels on selected carcass traits in capons
Daria Murawska, Tomasz Mieszczynski, Jan Jankowski, Michal Gesek

Dose response of a phytase on (phytate) phosphor, protein and ash digestibility and on bone ash in broilers
Saskia Leelu, Évelyne Delecize, Marta Lourenço, Lode Noisel, Magnus Jeremiasson

Effects of new generation plant extract mixture on quail performance and caecal microflora
Ece Çetin, Ismail Çetin, Derya Yesilbag, Seran Temelli

Effect of post-hatch feeding with hydrated diet supplemented with multi-nutrients on performance of broiler chicken
Yashar Rouhzendeh, Shirin Honarbaksh, Mojtaba Zaghari, Saeed Aminzadeh, Shokoufe Ghazanfari

Effect of fasting and early nutrition on morphology and microflora of intestine in broiler chickens
Reza Mirbak, Shirin Honarbaksh, Seyed Davood Sharifi, Saeed Aminzadeh

Identification and denombrement of some species of migratory birds vectors of avian influenza viruses
Nadir Alloui, Abdelhaq Barberis, Amine Boudaoud, Naoual Hamoudi

Oxidative and Microbiological Quality of Phosphate-Free Restructured Chicken Steaks During Frozen Storage
Burcu Öztürk Kerimoğlu, Meltem Serdaroğlu, Aslı Zungur Bastoğlu, Sibel Kaya Bayram

Effects of Using Broccoli Powder and Sodium Carbonate as Phosphate Replacers on the Quality Characteristics of Model System Chicken Emulsions
Burcu Sari, Hilal Can, Meltem Serdaroğlu

Polyphenols improved the meat lipid and sensory attributes affected by incorporation of fish meal as alternate protein source in broiler chicken diet

Comparative analysis of the microbiome associated to poultry carcasses from conventional and antibiotic free farms
Gerardo Manfreda, Alex Lucchi, Chiara Oliveri, Achille Franchini, Alessandra De Cesare

Scrutinizing mixer efficiency and poultry feed homogeneity
Omid Nouri, Mojtaba Zaghari, Hossein Mehrvarz

Evaluation of the effects of activated zinc oxide on the specification of broiler chicken bone
Hossein Mehrvarz, Mojtaba Zaghari
Eggs are sources of 18 vitamins and minerals, protein, fats and micronutrients that play an important role in basic nutrition. But because of the saturated fat content and cholesterol content during the past 40 years, the public had been warned against frequent egg consumption due to the high cholesterol content in eggs with possible potential association with Cardio Vasculare Disease (CVD). Based on this belief dietary recommendations in most countries have included dietary restrictions of egg consumption. Half a century of research have shown that egg and cholesterol intake is not associated with increased CVD risk. Consequently dietary cholesterol and egg restrictions have now been removed from most national dietary recommendations. Egg represent a very important food source, especially for some populations such as the elderly, pregnant women, children at graving age, convalescents and people who are sports training.

The history of the recommendations for eggs reviewed for 50 years starting from 1968.

Key words: egg, egg consumption, cholesterol, dietary guidelines, bioactive components, diabetes, atherosclerosis, nutrition

Introduction

Eggs are sources of 18 vitamins and minerals, protein, fats and micronutrients that play an important role in basic nutrition. But because of the saturated fat content (about 3 g/100 g) and cholesterol content (about 200–300 mg/100 g) during the past 40 years, the public had been warned against frequent egg consumption due to the high cholesterol content in eggs with possible potential association with Cardio Vasculare Disease (CVD). (Eilat-Adar et al. 2013)

In 1968, the American Heart Association (AHA) recommended the consumption of no more than 300 mg/day of dietary cholesterol and emphasized that no more than 3 eggs should be eaten per week, resulting in substantial reductions in egg consumption for everybody not just for coronary heart disease patients but also by healthy individuals. These recommendations did not take into account that eggs not only contain important nutrients for overall health but also components which exert protection against chronic disease. (Fernandez, 2016) Consequently people lost a highly nutritious food.

We will review the history of the recommendations for eggs, the components of eggs providing beneficial effects against disease, the relationship between egg intake and healthy eating index, the protective effects of eggs against inflammation and oxidative stress. Eggs have been recognized as functional foods due to the presence of bioactive components, which may play a role in the prevention of chronic and infectious diseases. (Miranda et al., 2015) The presence of antimicrobial, antioxidant, anti-cancer and hypotensive properties will be discussed.

Nutritional Value of Eggs:

It is widely recognized that eggs are a highly nutritious food based on their high quality protein and compliment of vitamins and minerals. Eggs are one of the most widely available economical sources of animal protein. It is more obvious when considering the nutritional needs of growing children, pregnant women, and the elderly. Because eggs are a conventional food containing nutrients that play fundamental roles beyond basic nutrition, their promotion as functional foods has been postulated for people of all ages and at different stages of life. In particular, eggs may play a particularly useful role in the diets of those at risk of low-nutrient intakes such as the elderly, pregnant women and children. (Natoli et al., 2007)

Phospholipids are among the bioactive components of eggs. Sphingomyelin and Phosphatidyl Choline have been postulated to regulate cholesterol absorption and inflammation and, interestingly, the incorporation of egg phospholipids into High Density Lipoprotein (HDL) appears to be a major factor in the cholesterol-accepting capacity of this lipoprotein. (Blesso, 2015) Ovoalbumin, a protein present in egg, is well known for its antioxidant properties. There is evidence that ovoalbumin and its peptides possess antiviral activity, as well as antioxidant and anti-inflammatoryatory properties. (Giansanti et al., 2015) In addition, egg yolk proteins including Vitellogenin, Lipovitellin and Phosvitin have also been shown to participate in the immune defense system, capable of killing bacteria and viruses as well as promoting phagocytosis activity. (Sun and Zhang, 2015) A study conducted in rats demonstrated that egg white protein was very useful for the recovery of iron-deficiency anemia. These roles of egg proteins in protecting against bacterial infection further document the association between egg consumption and health. (Kobayashi et al., 2015)

The anti-inflammatory properties of eggs have been demonstrated in numerous studies. Among the egg components with anti-inflammatory properties are: phospholipids, the carotenoids, lutein and zeaxanthin and egg proteins. The mechanisms of action of these anti-inflammatory components is discussed in detail. (Andersen, 2015) The components of eggs that have been shown to

Abstract

Plenary Session
There is controversy regarding egg consumption and patients diagnosed with diabetes. While it is clear that heart disease does not increase by egg ingestion, epidemiological studies have shown that egg consumption may have associated negative effects on CVD outcomes in patients with diabetes. A clinical study compared the effects of two distinct breakfasts in diabetic patients in a crossover design: one egg per day or 1 cup of oatmeal per day for 5 weeks each. The authors report that there were no differences in the parameters related to cholesterol or glucose metabolism between dietary interventions. However, following egg consumption, there was a reduction in liver enzymes and inflammatory markers in these patients. This study demonstrates that, for the specific population, egg intake did not increase cardiovascular disease risk but was rather protective against inflammation. (Ballesteros et al., 2015)

Recent systematic reviews found no clear relation of egg consumption and CVD among diabetic individuals. (Tran et al., 2014) I would like to review the fifty year of struggle starting from 1968 of the egg with the experience of Donald J. McNamara who is Director of Eggs for Health Consulting, in the United States. (McNamara 2015) According to McNamara, of the vast variety of foods that humans consume, only one has ever been specifically singled out for restriction in an effort to reduce CVD risk in the population as a victim; the egg. The most widely known dietary recommendation in the world is the 1968 admonition from the AHA to consume no more than three egg yolks per week. (Committee on Nutrition, American Heart Association, 1968) In 1968 who knew what 10% of calories from saturated fat or 300 mg cholesterol actually meant. For health professionals it was easy to explain dietary equivalence (high fat, high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol equals high cholesterol) without getting into detailed explanations of fat, calories, etc. The question many scientists raised was whether or not this recommendation would actually have any impact on CVD rates. But like the mistaken views in nutritional sciences, it was thought that it couldn’t hurt. But nobody never calculated that recommendation based on misunderstood data actually damaged the general public in terms of their nutritional needs and the egg industry also.

The egg industry was faced with a difficult situation starting from 1968: Unfortunately the dietary cholesterol phobia being promoted by the AHA. The other challenge, should the industry decide to finance research in an attempt to prove the innocence of the egg, “the industry funded research” “have often been nullified of the credibility of the results.” The decline in egg consumption in the US after the AHA recommendations were published, and further still after the 26 March 1984 Time Magazine cover prompted the US egg industry to establish the Egg Nutrition Center (ENC) to promote research and initiate health education efforts to address issues raised by the AHA in 1968, the Select Committee on Nutrition in 1977 and eventually the National Cholesterol Education Program” (NCEP) and Blood Institute of the National Institutes of Health (NIH). By the time the concept of low cholesterol was a confusion between dietary cholesterol and blood cholesterol. The evils of cholesterol, and eggs, were constantly presented to the consumer.

ENC formed a scientific advisory panel in 1984 composed of clinical and university researchers to help formulate a long range research strategy, as well as to serve as consultants to the industry and spokespersons on behalf of the industry. According to McNamara contrary to the “consensus” argument, not all nutritional scientists were convinced that the low-fat, low-cholesterol diet was the best answer to our CVD problems. He served on that original scientific advisory panel because he had carried out studies on the effects of egg intake on blood cholesterol levels and endogenous cholesterol metabolism and had serious doubts regarding the contribution of dietary cholesterol to blood cholesterol levels and CVD risk. (McNamara, 1985)

Epidemiological evidence that high dietary cholesterol was associated with high CVD incidence and epidemiological data in the 1960s and 1970s relied on simple correlation analyses to show associations and did not account for collinearity of nutrients (saturated fat and cholesterol found in animal products). Eventually multivariate analysis of dietary lipids and CVD incidence documented that dietary cholesterol was not an independent risk factor. (Hegsted and Ausman, 1988, McNamara, 2000)

Over the years the egg industry funded a variety of animal and clinical studies indicated that eggs had little effect on CVD risk. Retrospective analysis of existing clinical data also indicated that eggs and dietary cholesterol, when consumed at physiologic, not pharmacological levels, did not significantly affect CVD risk profiles. Retrospective review of epidemiological studies which involved analysis of egg intake showed that egg intake was not associated with CVD incidence. Epidemiological studies using multivariate analysis reported that dietary cholesterol was non-significant as an independent factor in CVD incidence. (Hegsted, McNamara, 1988) Based on these facts, the egg industry considered that it was justified in its efforts to challenge the egg restriction recommendation. By 1995 there was a concerted effort to unify all the US national dietary recommendations (so as not to confuse the public) such that the AHA, the NIH, the US Department of Agriculture, the US Department of Food and Drug Administration (FDA) all set the dietary cholesterol recommendation at less than 300 mg/day. (McNamara, 2015)

Since one large egg contained 213 mg of cholesterol, per capita egg consumption continued to be low. ENC continued to argue against egg recommendations and presented the scientific documentation showing no significant effect of eggs on CVD risk.

In 1995 the egg industry initiated a two pronged approach to dealing with the dietary cholesterol issue: detailed studies of the effects of egg intake on CVD risk factors and studies of the contribution of eggs to the diet of a healthy diet across the lifespan. ENC initiated research projects on a wide variety of themes to document why eggs should be included in the diet. Evidence that eggs contain other biologically active compounds that may play a role in the therapy and prevention of chronic and infectious diseases. The presence of compounds with antiviral, immunomodulator, antioxidant, anti-cancer or anti-hypertensive properties have been reported in eggs. (Abeyratne et al., 2013)

**Egg Protein and Satiation**: Egg protein, especially egg yolk protein, has a significantly greater satiety effect than other protein sources. Studies have shown after an egg breakfast greater weight loss over 8 weeks with larger changes in satiety hormones. One five decade long shift from eggs for breakfast to carbohydrate rich cereals probably contributed to “American national obesity problem”.

**Egg Protein and Sarcopeina**: There are a number of factors which can impact the dietaryavailability of high quality animal protein for elderly people; availability, affordability, ease to prepare, cooking and well digesting. Affordable sources of high-quality animal protein in the diet, eggs that are widely available are of significant importance for growth and development in children as well as for reducing the rate of sarcopenia and containing lean muscle tissue mass in the elderly. (Houston et al., 2008) Unfortunately hearing about the dangers of egg cholesterol, many old people studiously avoid eggs consuming, definitely to their detriment.

**Leucine**: Fifteen grams of egg white protein contain about 1300 mg of leucine and is also an abundant source of branched amino acids and aromatic amino acids. Leucine induces a maximal skeletal muscle protein anabolic response in young people, which suggests that egg white protein intake might have an important effect on body mass accretion (Hida et al., 2012) and stimulates skeletal muscle synthesis independently. Additionally, for people in sports training, egg proteins may have a profound effect on the development of muscles, but by its inclusion in the diet, it could be possible to enhance skeletal muscles synthesis (Herron and Fernandez, 2004) also leucine decreases muscle protein breakdown. (Glynn et al., 2010)

**Egg Xanthophylls, Lutein and Zeaxanthin**: Other interesting egg components from the nutritional point of view are the carotenoids. Carotenoids are natural pigments in hen egg yolks that confer its yellow color, which can range from very pale yellow to dark brilliant orange. Egg carotenoids represent less than 1% of yolk lipids, and are mainly composed of carotene and xanthophylls, lutein, zeaxanthin. (Skurray and Englaermova, 2014)Eggs provide highly bioavailable forms of the xanthophylls lutein and zeaxanthin which are related to lower risks for age-related macular degeneration and cataracts. Studies showed that egg lutein had high bioavailability and that adding eggs to the diet could result in significant increases in macular pigment optical density. (Wenzel et al., 2006)

**Egg Choline**: Choline plays an important role in fetal and neonatal brain development (Zeisel and Niculescu, 2006) and inadequate choline intake during pregnancy increases the risk for neural tube defects such as spina bifida (Shaw et al., 2004). The importance of dietary choline for brain development has been shown in numerous hypercholesterolemic and hypolipidemic animal studies with critical periods can have very negative effects. (Shapira, 2009) Adding an egg a day to the diet could alleviate this inadequacy. (Yonemori et al., 2011) Choline intake is also associated with decreased plasma levels of homocysteine and inflammatory factors, which are related to increased cardiovascular disease risk. (Detopoulou et al., 2008)

**Phosphitin**: It was suggested that egg-yolk phosphitin has the potential to be used as a natural bioactive compound as a hyperpigmentation inhibitor for human skin. (Jung et al., 2012)

**Egg and Cardiovascular Disease**: The egg industry also supported a series of studies looking at the chronic and acute effects of egg intake on endothelial function in a variety of patients and found no evidence of adverse effects with daily egg ingestion on any cardiac risk factors in normolipidemic and hyperlipidemic adults or in adults with CVD. (Katz et al., 2015, Katz et al., 2005)

In 1999 Hu et al. published one of the largest, long term population studies on egg intake and CVD incidence.2 The results from over 117,000 men and women documented no differences in CVD risk between those consuming one egg a week versus one egg a day.2 Other epidemiological studies have reported similar findings (ZSchwartz et al., 2007, Nakamura et al., 2006) Meta-analyses have come to the same conclusion. (Shin et al., 2013, Tran et al., 2014)

In 2002 the AHA dropped its specific egg restriction of 3 - 4 per week while keeping the less than 300 mg/day of dietary cholesterol guideline. It took another twelve years for AHA to pronounce that “There is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C.” (Eckel et al. 2014) In 2015 Dietary Guidelines Advisory Committee (DGAC) will not bring more to no more than 1 egg intake to be limited to a maximum of more than 300 mg total cholesterol, which is the relationship between consumption of dietary cholesterol and serum cholesterol, consistent with the conclusions of the AHA/ACC report. (Dietary Guidelines Advisory Committee 2015) A Recommendation known worldwide that lasted for 47 years has simply been discarded and we can all go back to including eggs in our diets.

Our research group in 2009 published an article. This research was to investigate the need to be concerned for the Turkish male population who is eating certain amount of eggs, and whether this consumption would increase the level of blood cholesterol and the incidence of CVD in this population. (Suvaci et al., 2009) The research was completed with 550 male subjects among from 15 to 59 and planned in two age groups (32 adult, 23 young) and four 30-days periods (egg free, 15 eggs/month, 30 eggs/month, 60 eggs/ month). The parameters checked (one baseline, and at the end of the three periods) in the blood samples were: Hemoglobin (Hb), Fasting blood glucose, Hemoglobin A1c (HbA1c), High Sensitive CRP (HsCRP), Lipoprotein a (Lpa), Homocystine, Triglycerides, Total cholesterol, HDL, LDL, VLDL. The results of this study suggest as well as in healthy male subjects, egg consumption of one whole egg/day has no adverse effect on the blood parameters related to increased cardiovascular disease risk. Interestingly both LDL and the total cholesterol levels seem either not to change at all or even fall a little with egg-consumption. On the other
Based on this belief dietary recommendations in most countries have included dietary restrictions of egg consumption. Half a century of egg consuming on the world.

**Table 1. Nutritional composition of hen eggs.**

<table>
<thead>
<tr>
<th>Component (Unit)</th>
<th>Amount</th>
<th>Component (Unit)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg shell (%)</td>
<td>10.5</td>
<td>Calcium (mg)</td>
<td>56.0</td>
</tr>
<tr>
<td>Egg yolk (%)</td>
<td>31</td>
<td>Magnesium (mg)</td>
<td>12.0</td>
</tr>
<tr>
<td>Egg white (%)</td>
<td>58.5</td>
<td>Iron (mg)</td>
<td>2.1</td>
</tr>
<tr>
<td>Water (g)</td>
<td>74.5</td>
<td>Phosphorus (μg)</td>
<td>180.0</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>162</td>
<td>Zinc (mg)</td>
<td>1.44</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>12.1</td>
<td>Thiamine (mg)</td>
<td>0.09</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>0.68</td>
<td>Riboflavin (mg)</td>
<td>0.3</td>
</tr>
<tr>
<td>Lipids (g)</td>
<td>12.1</td>
<td>Niacin (mg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Saturated fatty acids (g)</td>
<td>3.3</td>
<td>Folic acid (μg)</td>
<td>65.0</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (g)</td>
<td>4.9</td>
<td>Cyanocobalamin (μg)</td>
<td>66.0</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (g)</td>
<td>1.8</td>
<td>Pyridoxine (mg)</td>
<td>0.12</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>410</td>
<td>Retinol equivalents (μg)</td>
<td>227.0</td>
</tr>
<tr>
<td>Iodine (μg)</td>
<td>12.7</td>
<td>Potassium (mg)</td>
<td>147</td>
</tr>
<tr>
<td>Tocopherols (μg)</td>
<td>1.93</td>
<td>Carotenoids (μg)</td>
<td>10</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>10</td>
<td>Cholecalciferol (μg)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Quantities represent an edible portion of about 100 g. (Miranda, et al., 2015)

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MIRANDA, J.M., ANTON, X., REDONDE-VALBUENA, C., ROCA-SAAVEDRA, P., RODRIGUEZ, J.A., LAMAS, A.,


Abstract

Humans are a typical omnivore species. Anyway, meat consumption varies distinctly between cultures and stage of development (in the view of economy) of countries. Highly developed countries are typically eating more meat than low developed ones, but in highly developed countries eating habits (food styles) are changing distinctly since the beginning of the 21st century. People are more and more concerned about the way of animal production and thus change to flexitarian, vegetariand and vegan eating habits. An increasing number of people is also interested in artificial meat (analog meat), insect protein and cultured meat. Especially, cultured meat (clean meat) is believed to have the potential to replace ‘normal’ meat in the human menu to a distinct degree, despite many unsolved questions. Clean meat is attractive as it does not require the keeping of farm animals and as the production is believed to have a lower impact on the environment. Therefore, clean meat may become a serious competitor of ‘normal meat’, but this may take more time than currently expected.

Introduction

Consumption of animal products (mainly meat) was the basis of the development of the cognitive capability of humans. This made the tremendous development of the human species within a few millennia possible. Anyway, the share of meat in human food evolved differently in different regions of the World, mainly due to cultural and/or religious constraints. In Asia or Africa, e.g., vegetables comprise (still) a distinct portion of the daily food intake, traditionally. Besides, in these continents, pork and beef meat consumption is rather low, whereas, poultry meat is accepted as food and consumption is continuously increasing. In general, meat consumption is closely connected with the status of prosperity. Therefore, consumption of animal protein is increasing globally due to the development of the economy during recent years, especially in Asia. Due to the high number of people living in this continent an increase in meat consumption by 1.0 percentage point results in a huge increase in the overall production. Globally, poultry production increased by about 20% within the last 5 years, mainly due to the increase in consumption in Asia, and reached about 120 million tons of slaughter weight in 2017 (Figure 1). But, the highest per capita poultry meat consumption still exists in ‘old’ developed (= highly industrialized) countries (Table 1).

Despite the still high meat consumption in the ‘old’ developed countries more and more people are changing their eating habits (food styles) to a more vegetable or even animal products free (vegan) food. This is mainly caused by social changes. On the one hand people are more and more dealing with animal welfare and the other hand they are seeking a more healthy nutrition. People missed the development in poultry production towards huge, highly engineered production units and thus are shocked by today’s status of ‘mass production’. This feeling is intensified by the continuous development of a more emotional relationship towards animals, what is typical for countries with a high living standard. People have lots of time and do not have to struggle for income any longer, leaving plenty of room for alternative activities, including the keeping of pets. On this background, the eating behavior of people has diversified during recent years. The spread is now between dominantly carnivorous to totally vegan.

Thus, the present overview attempts to summarize the probable reasons behind this development, to describe the present situation and to give an estimation for the future development and its impact on poultry meat production and/or consumption.
Table 1. Chicken (kg/head/year; MEG, 2018)

| Country    | 2017 | EU-28  | 19.4 | Russia, Federation | 27.5 | Brazil | 44.2 | USA    | 48.0 | China  | 8.3 | India  | 3.3 |

Table 2. Human eating habits in 2016 (Statista, 2018)

<table>
<thead>
<tr>
<th>Habit</th>
<th>Asia</th>
<th>USA/EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexitarian</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Vegan</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
human food are approved within the European Union under considering strict hygienic protocols (Novel Food Regulation (EU) 2015/2283). So far, the use of excreta or food waste as substrate is not allowed, but the latter one is under discussion. As mainly people in industrialized countries are sick at insects this type of alternative protein food will probably not gain a bigger market share. It will remain in a niche as adventure food.

Replacement meat or analog meat is not a real new approach. Many attempts have been undertaken to produce ‘meat like’ products on a solely vegetable basis. This was also the beginning of the vegetarian era. The problem here is that consumers refuse to eat real meat for many reasons, but they demand, nevertheless, the same sensory attributes for the ‘artificial’ meat products, including sausages. This is also reflected in product names – ‘veggie burger, veggie sausage etc. – a clear contradiction! In many cases lupin or pea protein is used as a basis and sensory attributes are achieved by supplementing herbs and spices (VZHH, 2014). Nevertheless, despite a similar taste to meat burger or sausages the texture is different (Windhorst, 2018b). In addition, a new variation of replacement meat are novel protein foods like soybean meat, quorn, milk schnitzel or insect burger. Anyway, a distinct share of vegetarians and vegans are purchasing these products, but this share will probably increase only slowly.

Clean meat, also known as laboratory-cultured meat, cell-cultured meat or in vitro-meat, is a rather new product with a promising future. This idea started with its realization in 2013 and the procedure meanwhile could be developed in a way that an area-wide production seems to be possible (Windhorst, 2018b; Deutscher Bundestag, 2018; Windhorst, 2019a). To cultivate muscle cells stem cells from calf fetuses are collected and transferred to a nutrient solution. The cells are multiplying to a muscle cell layer which is harvested and processed to burger or similar products (Stephens et al., 2018). Probably, the production of a real meat like a steak will not be possible. As only few companies are doing research in this field, information on the composition of the nutrient solution and the harvesting process of stem cells is scarce. The main advantages of clean meat are: no killing of animals, less environmental pollution, less use of feed and water, no use of drugs (e.g. antibiotics), fast adaptation to changing consumer demands. But, there are still many open questions (Stephens et al., 2018; Thorrez and Vandenburgh, 2019). Is it valid to use the term ‘meat’ for this product? Can this product also be eaten by vegetarians and vegans? Furthermore, still a lot of problems have to be solved (Windhorst, 2018b).

Where do the components of nutrient solutions come from? Have stem cells to be genetically modified? Is it ethically justifiable to use a fetus as donor for stem cells? How many fetuses are required? Are they still available after reducing the number of animals kept on farms? What happens to the animal keeping farms? How can the shortfall of manure be compensated? How much protein is necessary to produce 1 kg muscle tissue? Where are the protein feed stuffs grown? Is clean meat only a product for developed countries? Can clean meat really replace ‘conventional’ meat production with animals? Do consumers accept the deviating sensory attributes? Will the consumer accept the use of stem cells? Despite these open questions, there are several benefits for companies to develop clean meat (Windhorst, 2018b): ethical reasons (no keeping and killing of animals), improvement of image (no cruelty due to killing of animals), enlargement of product portfolio, being a pioneer in this new market, being the first in the market. This is also the reason for huge companies like Tyson Foods or Wiesenhof to invest in this technology. In summary, clean meat is a new approach to produce ‘meat’ in an ethical way. The term ‘cellular agriculture’ has been defined for this way of production, meanwhile (Windhorst, 2019b). This development is supported by the market as people are more and more refusing to exploit animals for their own benefits. Especially, meat production has to be seen critically due to the necessity to kill the animal before harvesting the meat.

The possibility to produce cultured meat has created a new eating habit – neomorphism (Windhorst, 2019b). It is hard to estimate the market potential of this alternative meat product. Despite the product price will be reasonable until 2020 it will take another 5 to 10 years to extend the production and to develop the market (Stephens et al., 2018). Although it is unlikely that clean meat will replace ‘real’ meat within a short time, in a medium perspective it has the potential to acquire a significant market share. This is mirrored by the increasing number of companies dealing with the production of clean meat (Table 3). In a long-term perspective, replacement of real meat by clean meat in developed countries is imaginable.

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Just, Inc.</td>
<td>San Francisco, CA</td>
<td>Chicken</td>
</tr>
<tr>
<td>Memphis Meats</td>
<td>Berkeley, USA</td>
<td>Beef, poultry</td>
</tr>
<tr>
<td>Supermeat</td>
<td>Tel Aviv, Israel</td>
<td>Chicken</td>
</tr>
<tr>
<td>Aleph Farms</td>
<td>Ashdod, Israel</td>
<td>Beef</td>
</tr>
<tr>
<td>New Age Meats</td>
<td>San Francisco, CA</td>
<td>Pork</td>
</tr>
<tr>
<td>Meatable</td>
<td>Leiden, Netherlands</td>
<td>Beef, pork, chicken</td>
</tr>
<tr>
<td>Higher Steaks</td>
<td>London, UK</td>
<td>?</td>
</tr>
<tr>
<td>Mosa Meat</td>
<td>Maastricht, NL</td>
<td>Beef</td>
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</tbody>
</table>

**Conclusions**

Mainly, in the old industrialized countries people are more and more dealing with the way of producing and processing of their food. This holds especially for animal products, i.e. meat. People believe that the way of production is in conflict with animal welfare and causes environmental pollution. Therefore, a significant portion of consumers changes their eating habits towards flexitarian, vegetarian and/or vegan nutrition. This paves also the way for new foods like analog meat and cultured meat. Especially, cultured meat has the potential to capture a significant portion of the ‘meat food’ sector and may become a serious competitor to conventional meat. This will not happen on a short-term but probably on a medium term.

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Novel egg components and human health

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Abstract
Egg contains many macronutrients and micronutrients that have been shaped during evolution to support self-sufficient development of the embryo, until hatching. Eggs gather a diversity of compounds that are of particular interest for human nutrition while offering a moderate calorie source. The perfect balance in its nutrients together with their high respective digestibility has put the egg in the spotlight as a basic food for humans. In addition, the egg is believed to contain a myriad of biologically active components. Most of these components are not fully characterized yet but some of them are predicted to be of high interest in preventing/curing some inflammatory and infectious diseases. These components are concentrated in the various edible egg components that mostly include the egg white and the egg yolk together with its tightly associated vitelline membranes. Several activities such as antimicrobial and antioxidant properties have been reported for major egg white proteins like ovallubumin, ovotransferrin and lysozyme, but also from their derived hydrolytic peptides. Interestingly, some of these bioactive peptides may be generated naturally in vivo during the process of digestion, to participate in gut health and homeostasis. However, there is currently a lack of knowledge regarding the fate of egg proteins in the intestinal tract and the biological significance of such molecules. Considering the several hundreds of proteins that have been identified to date, the egg is likely to contain many additional nutraceuticals and peptides that need to be further characterized. In fact, there are increasing data illustrating the bioactive potential of egg components of low abundance. This review will give an overview of current data on egg bioactive compounds and will propose some emerging research on egg-derived nutraceuticals.

Keywords: egg, chicken, nutrients, bioactivities, hydrolytic peptides, nutraceuticals

Introduction
There is increasing evidence that egg is not solely a basic food of high nutritional value that is extensively consumed worldwide, but that it also contains many bioactive compounds including proteins and derived hydrolytic peptides of major interest for human health. In vitro analyses performed on purified proteins and on hydrolytic peptides (generated after limited proteolysis by digestive proteases) have revealed a great potential in these molecules as nutraceuticals. Nevertheless, the egg contains many molecules whose biological activities remain unexplored, which offers promising researches in the field of bioactive compounds. A total of 1261 non redundant protein sequences (gene products), has been identified by proteomics (Gautron et al., 2019). Interestingly enough, most egg proteins and peptides that have been identified by proteomic approaches have no associated physiological function yet. Indeed, several of them lack homologs in mammalian species and their biological/physiological activities have not been characterized so far. Various in silico tools are now available to predict biological activities of peptides and proteins to overcome this lack of functional data associated with proteins. Recently, these bioinformatics approaches have been applied to yolk to identify peptides with putative anti-inflammatory, antimicrobial, anticancer, antiviral, antibiofilm, calcium-binding, and anti-inflammatory activities (Arena and Scabini, 2018).

In literature, we can find numerous publications describing the antimicrobial activities of egg-derived proteins and peptides. In addition to antimicrobial egg proteins that are naturally present in the egg, there is compelling evidence that some hydrolytic peptides with additional activities may naturally appear during the digestive process of egg proteins. Interestingly, some of these bioactive peptides are specifically generated after limited proteolysis of denatured egg proteins, a process that is accelerated by heating/boiling, while others are produced following partial degradation of raw egg compounds. Although information on the fate of egg proteins in the digestive tract is limited, it is known that some egg proteins (ovallubumin, ovomucoid) are only partly digested, which suggests that some bioactive peptides may be generated naturally without undergoing complete degradation into free amino acids. From these results, it can be assumed that some of these bioactive peptides (antimicrobial, antioxidant, immunomodulatory molecules) can participate in gut health and homeostasis (Figure 1). Altogether, these data highlight the potential use of these molecules as additive ingredients for the development of functional foods. The exhaustive list of the biological activities associated with egg proteins or derived peptides have been updated recently, in two complementary publications (Rehault-Godbert et al., 2019). In this report, a special focus will be made on egg proteins that may contribute to gut health and homeostasis.

Bioactive compounds

Antimicrobials
Egg antimicrobials in edible parts are essentially concentrated in egg white and the vitelline membrane, although the eggshell also contains many antibacterial proteins of minor abundance including some that are specific to the eggshell (ovocalyxins). As the eggshell is removed before consumption by humans, we will not discuss eggshell compounds in this review but information on these molecules is available elsewhere. Egg antimicrobial activities encompass antibacterial, antiviral, antifungal or antiparasitic activities (Guyot et al., 2016; Herve-Grepinet et al., 2010; Rehault-Godbert et al., 2011; Gautron et al., 2019). Their bacteriostatic and bactericidal activities rely on two main mechanisms. The effect of some antimicrobial egg proteins can be triggered by the direct interaction with microbial walls, which subsequently destabilize and permeabilize bacterial structures (lysozyme, avian beta-defensins, etc.) leading to microbial lysis and death. The effects of the other molecules are rather indirect by decreasing the bioavailability of essential nutrients for the pathogens such as iron (ovotransferrin) and vitamins (avidin). Egg also contains high amounts of antiproteases that are also likely to participate in egg defense. Indeed these molecules (ovoinhibitor, cystatin) may constitute potent inhibitors of microbial proteases that are virulent factors of infection. This aspect is also quite interesting as some protease inhibitors (essentially in vegetables) are described as defensive molecules that discourage consumption by predators (by interfering with digestive proteases thus altering digestion efficiency) (Mohranji et al., 2018). It is also noteworthy that some egg antimicrobials are specific to Bird species and are of major interest for Public Health to be used as alternatives or in combination to antibiotic therapy. Indeed, their original/specific three-dimensional structure and/or net charge that may explain their respective mechanisms of action and their antimicrobial spectrum of activity, can be used as a template to develop and to design potential alternatives to conventional antibiotics. Knowing that some of these molecules are cysteine-rich, a property that generally confers high resistance to proteolytic degradation and denaturation (avian beta-defensin 11, beta-microsenameinprotein, ovomucoid and ovoinhibitor), we hypothesize that these molecules may be recovered in the intestinal tract in their native form to contribute in reinforcing intestinal immunity of egg eaters. In addition to these egg proteins, there are also numerous data on egg-derived peptides that may be released after partial hydrolysis by digestive or exogenous proteases. Such hydrolytic peptides obtained from lysozyme, ovotransferrin, ovomucin and from cystatin have shown a broad range of antibacterial activities.

Antioxidant activities
It is well assumed that long-term oxidative stress can lead to chronic disorders and there is increasing interest in investigating the potential of food-derived compounds bearing antioxidant activity. Chicken egg contains many antioxidant proteins, either in their native form or as derived peptides, such as ovotransferrin, ovomucoid, ovomucin and yolk phosphitin. Most assays reporting such activities are in vitro assays but some experiments performed in a porcine model have revealed the beneficial effect of proteins derived from egg yolk, in reducing the production of pro-inflammation cytokines (Young et al., 2010). The authors concluded that supplementation of the diet with egg yolk proteins, may be a novel strategy to reduce intestinal oxidative stress.

Anti-cancerous molecules
There are only few data showing that food-derived proteins and peptides can also be beneficial to treat cancer diseases. Several studies have confirmed the tumor-inhibitory activity of egg white lysozyme via its immunopotentiation properties (Sava, 1989). Ovomucin (beta subunit) and ovomucin-derived peptides also showed anti-tumor activities by combined cytotoxic and immunostimulating effects. The anticanerous effect of hydrolytic peptides from ovotransferrin (Ibrahim and Kiyono, 2009) has also been published. But, in general, information in this field is quite scarce and deserves further investigation.
**Immunomodulatory activities**

Egg white lysozyme has been shown to be a promising agent for the treatment of inflammatory intestinal disease such as colitis, by reducing the local expression of pro-inflammatory cytokines while increasing the expression of the anti-inflammatory mediators (Lee et al., 2009). In human, pleiotrophin, which has a chicken homolog that is present in the egg, has been shown to promote lymphocyte survival, and to drive immune cell chemotaxis (Sorrelle et al., 2017). Immunomodulatory activities might also emerge from ovotransferrin and egg yolk vitellogenin, and ovumucin hydrolysates (Liu et al., 2017; Wang et al., 2017) after partial degradation by digestive proteases. For most peptides, there is a lack of in vivo experiments that could confirm their benefit, to control digestive inflammatory disorders.

**Anti-hypertensive activities**

Hypertension is a multifactorial disease, where the renin-angiotensin-aldosterone system plays a major role. Most egg-derived peptides with anti-hypertensive activities exhibit inhibitory activities against the angiotensin-converting enzyme. This enzyme triggers the processing and activation of angiotensin I into the active vasoconstrictor angiotensin II. Several yolk-derived peptides bearing anti-hypertensive activities have been described in the literature (Yoshii et al., 2001; Yosu and Howell, 2015) but also ovotransferrin and egg white hydrolysates containing tripeptides (Majumder et al., 2015). The oral administration of these peptides in hypertensive rats was reported to significantly reduce blood pressure (Majumder and Wu, 2011).

**Impact of storage and cooking on the bioactivity of egg proteins and peptides and proteins**

There are several factors that may affect the intrinsic biological activities of proteins extracted from eggs: the genetic origin (presence of polymorphisms and abnormal splicing of genes that impact their respective protein function), storage, freezing and heating, pH, etc. Here, we will give a quick overview on the effect of storage and cooking/heating on egg proteins.

**Impact of storage**

The conditions of egg storage induce deep physicochemical modifications of internal egg compounds that may subsequently alter the biological properties of egg white (Berardelli et al., 2008; Guyot et al., 2016). These egg modifications result from water exchange between the yolk and the egg white and from water and carbon dioxide loss through eggshell pores. The value of egg white pH increases to more than 9.5 during storage. This alkalinisation of egg white pH may also negatively affect the biological properties of egg white (Liu et al., 2008). Here, we will give a quick overview on the effect of storage and cooking/heating on egg proteins.

**Impact of cooking**

Upon cooking, egg proteins undergo major conformational modifications, even though their relative content is not affected. This protein denaturation may be beneficial to inactivate antimicrobial factors such as egg white protease inhibitors that may interfere with the proper protein digestion. Heating also helps in denaturing highly resistant proteins, thereby facilitating their proteolysis in the digestive tract. A further digestibility of egg proteins may also contribute to limit hyposensitivity to eggs for children (Zha et al., 2018). Nevertheless, proteins denaturation is usually paralleled to the loss of their intrinsic biological activities. For example, it has been shown that cooking significantly reduces the oxygen radical scavenging capacity (antioxidant potential) of egg yolk (Remanan and Wu, 2014). These observations corroborate that taking into account the food matrix and the way eggs are cooked, is of major importance for the gastrointestinal health benefits (Nymth et al., 2016). To conclude, the beneficial-risk balance of cooking egg for human health is quite difficult to appreciate as many egg proteins may be affected by cooking, while in parallel the heating process increase the overall digestibility of egg proteins, and may potentially reveal new bioactive peptides. However, it is worth mentioning that cooking eggs allow to eliminate most potential pathogens (Salmoneilla bacteria), which is responsible for foodborne diseases.

**Conclusions**

Besides basic nutrients, eggs are a valuable source of potential nutraceuticals. With regard to the high number of egg proteins and peptides that have been identified in the edible parts of the egg, the egg encloses many unknown biological activities that merit further investigations. There is currently a lack of data reporting the fate of egg proteins along the digestive tract, which limits our perception of the benefits of egg proteins and of the resulting hydrolytic peptides in gut health. However, the availability of dynamic gastric models (Do et al., 2016; Thuenemann et al., 2015) may help in developing egg-based functional food. These in vitro models mimic both the biochemical and mechanical aspects of gastric digestion. They include artificial saliva, compressive forces to disintegrate food, and gastrointestinal secretions that generate pH profiles that resemble those found all along the digestive tract. In these models, bile salts and intestinal enzymes are acting in a realistic time-dependent manner, and such approaches may be improved in the future by adding intestine-like microbiota. This model was already used to assess the bioaccessibility of food nutrients (but has not been applied to the egg, to our knowledge) but also to study the structural changes of food matrices. Such an experimental strategy would constitute a promising way to study and model the impact of egg preparation (raw versus cooked) on the physiological generation of bioactive peptides (antimicrobial, antioxidant, immunomodulatory, anti-inflammatory but also anticancerous), and to better appreciate their biological significance for human health. It might be also very useful to better appreciate whether the digestive environment (pH, salt, proteases) can denature all egg proteins and peptides to generate bioactive hydrolitic peptides or whether some egg molecules resist denaturation and retain their activity in the intestine (cysteine-rich antimicrobial molecules and ovomucins). In addition to improving gut health, it is expected that such functional egg-derived compounds may also be beneficial to the human health in general, as it is now well assumed that gut health also contributes to normal brain function and mental health (Nicholson et al., 2012).

**References**


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**XVIII European Symposium on the Quality of Eggs and Egg Products**

**Invited Speakers**
In the laying hen industry, egg quality traits remain critical to the future. Eggs compete with many other foodstuffs in the market place. Although eggs have advantages over other products, it is unwise to be complacent. One of the major challenges that the industry is facing is the move towards a longer laying period (Bain et al., 2016, Presinger, 2018). To achieve this, breeders of laying hens will need to focus on a range of traits but egg quality will undoubtedly remain at the heart of the selection programme. Most specifically maintaining it over a much longer period. What then are the opportunities in terms of egg quality in research and in practice? I am going to use this opportunity not to simply review the past, but to suggest an agenda for the future.

1) New phenotypes; Although egg shell strength measured by puncture tests or quasi static compression and Haugh units remain the main egg quality traits, probably because of their relative ease of measurement, there remains considerable scope to develop new methods and new traits. Measurements of traits that have importance and are to date, not measured for genetic improvement are new methods of measurement. However, many of the measurements are currently time consuming to adopt and are not possible to use in practice. Innovative approaches to these traits are required. There is clear potential for the adoption of technologies to evaluate the vitelline membrane, which is important for the egg processing industry, the protein content of egg white for use in processing, the shell membrane thickness to improve translucency and the cuticle for preventing bacterial entry. These all traits where there is need for technical solutions and novel ways of approaching measurement.

Starting with the vitelline membrane, the fact that attempts have been made for almost 80 years to measure it suggests it is important (Moran, 1936, Scholtysek, 1995, Marzec et al., 2016). Its integrity is important to the processing industry but methods suitable for application in genetic selection have rarely been described. However, a simple method might offer some promise. Yolk index is correlated with vitelline membrane strength, however studies to date have only looked between different ages and species (Kuranda and McKee, 2000, Marzec et al, 2016). There are clearly opportunities for these studies to be made.

I believe egg albumen, the egg white, is a topic that also requires further investigation, Haugh units or simply albumin height are not used despite the fact that it is not necessarily a good representation of protein content. There is indeed evidence that Haugh units does not associate with the properties desired by the baking industry, a major market for eggs (Silverdides and Budgel, 2004) and albumin height, a component of Haugh units, is negatively correlated with hatchability (Cavero et al., 2011). Although the negative effect size was small, it might influence hatchability. More research is needed to understand the genetic correlations and how the traits would influence quality for processing as well as appearance as well as the effects on hatchability it may be worth examining further (Cavero et al., 2011). It is certainly a tenable hypothesis that protein content or quality may be a more reliable indicator of egg white quality and the availability of techniques such as FTIR (Panuszek et al., 2012), although difficult for aqueous samples, are constantly improving (Zhao et al., 2018). Alternative approaches comparing measurements of the wet and thick albumen by their degree of spreading with Haugh units or albumin height indicated a close relationship (Gao et al., 2018). In terms of the total spreading area, genetically this was largely determined by the degree of spreading of the wet albumen (Gao et al., 2018) and when the top and tail of the distribution was compared the eggs that spread least did contain the highest dry matter content, it was stated a negative genetic correlation between colour of these white eggs and the spreading ability, darker eggs spreading more, a situation not observed for Haugh units in brown eggs (Shao et al., 2005). Some of these trait measurements may well have application if the industry can wean itself from albumin height or Haugh units and favourable correlations are demonstrated.

The shell membrane has received minimal attention in terms of genetic parameters, yet it is the structure on which the shell is laid down and the nucleation of crystal growth is an important factor in the shell structure (Rodriguez-Navarro et al., 2015). In white shelled eggs particularly, a problem occurs called translucency when areas of the shell become translucent which is visible on penetration of the egg (Wang et al., 2017) as it was thinner in eggs displaying the trait, while the shell thickness was actually greater in the same eggs. The shell membrane has received minimal attention in terms of genetic parameters, yet it is the structure on which the shell is laid down and the nucleation of crystal growth is an important factor in the shell structure (Rodriguez-Navarro et al., 2015). In white shelled eggs particularly, a problem occurs called translucency when areas of the shell become translucent which is visible on penetration of the egg (Wang et al., 2017) as it was thinner in eggs displaying the trait, while the shell thickness was actually greater in the same eggs.

Finally, the cuticle, which has now repeatedly been shown in a range of breeds protects the egg from bacterial penetration (Bain et al., 2019, Bain et al., 2013, Kulshehradha et al., 2018, Chen et al., 2018). We understand more about how environmental factors influence the cuticle (Bain et al., 2017) and its relationship with shell thickness (Bain et al., 2019, Wilson et al., 2017). Cuticle deposition, unlike colour, does not appear to reduce with age in our studies (Bain et al., 2019). Delivering a simple measurement with good heritability means this trait will be improved with particular benefits for the hatching egg industry by reducing horizontal and vertical contamination of eggs (Dunn et al., 2019). The external colour and appearance of an egg seems to be largely of aesthetic and marketing, with different individuals and populations having different views on what they buy and find attractive. The understanding of the genetics of the colour of eggs and its determination has made considerable progress particularly in understanding of the genes (Zeng et al., 2014) but colour measurement is simple and quick and there needs little requirement to replace current reflectance based measurements. Other measurements of aesthetic appearance areas such as the shininess or smoothness of eggs is an area relatively little investigated (Igic et al., 2015, Icken et al., 2013) and which could be used if consumer preference was established.

2) Genomic selection; Traditional selection is not replaced by genomic selection; however it is already an important part of the approach breeders are taking. In the laying hen sector, the biggest advantages probably lie in the selection of males using the sib’s genetic prediction (Presinger, 2010, Albers, 2010, Wolc et al., 2016, Wolc, 2014). Increasingly that genomic prediction, using the genotype of the male, rather than his sisters performance will feature traits measured late in production. Again supporting the drive to move towards a closed breeding cycle over its longer laying period. Studies demonstrating the application of large sets of genetic markers exist. These both illustrate the techniques for application of genomic selection and highlight genomic regions controlling the traits. So for example how many generations of phenotype is best to train the data (Weng et al., 2016) or identifying chromosome regions explaining relatively large amounts of variation for albumen height, puncture score, yolk weight and shell colour (Wolc et al., 2014), or for egg shell weight and shell strength (Liu et al., 2011). Studies looking at selection in layers identified regions of large effect on albumin height and shell colour (Liu et al., 2018) and looking at other traits defining interactions of gene by diet on quality traits (Rome et al., 2015) identified regions for shell strength, albumin characteristics and colour across both diets (Rome et al., 2015). Although many genes with plausible credentials credits for controlling the traits exists in these studies, proof of their involvement remains to be established.

3) New breeding technologies; Editing the chicken genome is possible and lines of chicken have been created for scientific research (Tait-Burkard et al., 2018, Woodcock et al., 2017) as well as transgenic lines (McGrew et al., 2004, Herron et al., 2018). What, if any, role will this technology play in the future of the egg laying industry? In the European Union, recent judgements mean that there are obstacles to the adoption of the technology (Callaway, 2018). Currently there are transgenic lines for embryo and disease research and edited animals provide sterile hosts for the regeneration of cryopreserved primordial germ cells (Woodcock et al., 2017) but quantitative traits are a different matter. It is difficult to see that our knowledge is advanced sufficiently to tackle quantitative traits such as related to egg traits. Whilst we know the role of individual genes, we do not know what other traits they effect. Indeed, we have examples of genes that have beneficial effects on the cuticle but the opposite effect on shell thickness and shape (Dunn et al., 2015). However, conceptually it is possible and in the future applications for laying hens in the area of welfare may be especially attractive, but it may be some years until application to egg quality. In the meantime, there are opportunities for the production of eggs to supply valuable proteins and the chance to explore the biology behind some of the genes that control egg quality using these approaches.

To conclude; there are many new opportunities at the interface between chemistry or physics and biology to deliver phenotypes to support future genetic selection for improving or maintaining egg quality. This will be enhanced by the power of genomic selection to increase selection efficiency. The prospect of general editing is here, but currently it is less clear if that will have application for egg quality given the complexity of the trait, but progress in genomics is rapid.

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References:


Eggshell contamination and risk for food safety

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Abstract
The safety of food including eggs is a high priority in most countries. Egg contents may be contaminated by vertical transmission, where harmful micro-organisms present in the body of the hen enter the egg as it is forming in the ovary and/oviduct. Alternatively, an egg may become contaminated by horizontal transmission, where micro-organisms on the outside of the shell enter the egg after it has been laid by the hen. A number of factors influence the risk of contamination of egg contents. These include the disease status of the flock (influenced by type of production system and farm management), the processes of egg collection, handling, storage and processing and the ability of the eggshell and other components of the egg to withstand microbial ingress. The way in which the consumer handles the eggs also plays a part. Because the role of the hen’s egg in nature is as an incubation chamber for a developing chick, the egg has many properties that guard against microbial attack, in order to ensure the safety of the developing chick. These same properties also protect consumers of eggs and egg products. For organisms such as Salmonella enterica Enteritidis, which are transmitted vertically, emphasis needs to be on maintaining flocks that are free from the pathogen. This can be achieved by a combination of excellent biosecurity, provision of salmonella free feed, use of prebiotics and probiotics and, where appropriate, vaccination of the flock. For most other organisms, including others of type of Salmonella, which enter the egg via the horizontal route, emphasis needs to be not only on reducing the incidence of Salmonella in the flock, but also in enhancing the resistance of the egg to bacterial ingress and maintaining processes of egg collection and handling that minimise the opportunity for bacteria to be present on the outside of the egg and to enter the egg contents.

Key words: eggs, laying hens, food safety

Introduction
The safety of food including eggs is a high priority in most countries and eggs are regularly implicated in cases of food-borne illness. For example, as recently published Annual Report from the Australian OzFundNetwork (OzFundNetwork, 2018) lists eggs as being involved in 31% of foodborne disease outbreaks caused by known food vehicles during 2012, representing 19% of all foodborne outbreaks. 42% of foodborne Salmonella outbreaks (S. Typhimurium – the main organism of concern in Australia) and 51% of all outbreaks attributed to a single food ingredient. S. Enteritidis is not endemic in Australian layer flocks and, in the small number of cases where transmission has occurred from personnel returning from overseas, all eggs produced are recalled. In 2017, Salmonella outbreaks from eggs and egg products in Europe were 36.8% of total Salmonella outbreaks (31.3% of S. Enteritidis outbreaks) and were attributed mainly to S. Enteritidis and S. Typhimurium (EFSA and ECDCC, 2018).

Routes of contamination of shell eggs
Bacteria enter the hen’s egg by one of two basic routes (Gantois et al., 2011; Jan and Baron, 2017). Vertical transmission occurs when bacteria are transmitted from the hen into the egg from either the reproductive tissues or from the faeces during or just after oviposition. The organism associated most commonly with vertical transmission into eggs is Salmonella enterica serovar Enteritidis although other serovars such as Heidelberg and Typhimurium have been identified as being transmitted vertically (Gast, 2006a). A recent study in the U.S. found that egg sweating did not increase the risk of bacterial ingress into the egg and that more frequent collection potentially reducing the opportunity for contamination from the immediate environment. Maintenance of microbiological cleanliness of egg collection and handling equipment also reduces the risk of contamination.

Control of egg collection and handling
Many countries have codes of practice, voluntary or compulsory, which govern the stages of egg collection and handling. In Australia, the AECL (2010a) Code of Practice for Shell Egg, Production, Grading, Packing and Distribution is a voluntary code although compulsory codes exist in some countries. The Australian Eggs website (Australian Eggs, 2019) has a poster describing best practice in “Egg collecting, grading, washing and packing”. Frequency of collection of eggs can influence eggshell contamination, with more frequent collection potentially reducing the opportunity for contamination from the immediate environment. Maintenance of microbiological cleanliness of egg collection and handling equipment also reduces the risk of contamination.

Control of egg processing and storage
The processing and storage of eggs following collection affect egg contamination. All equipment needs to undergo regular effective cleaning. The Australian Eggs website (Australian Eggs, 2019) also has a poster on “Egg storage and transport”. Egg refrigeration
In some countries, objection to compulsory refrigeration of eggs has been based on the concern that “egg sweating”, arising when eggs are moved from a cooler to a warmer temperature, may facilitate the movement of bacteria on the shell surface into the egg contents. Results from earlier studies had indicated that condensation increased the penetration of Salmonella Enteritidis through eggshells when using the agar egg method but had no effect on contamination of egg contents in intact eggs (de Reu et al., 2006a). A recent study in the U.S. found that egg sweating did not increase the risk of bacterial ingress into the egg and that refrigeration was effective in inhibiting the growth of Salmonella Enteritidis (Gradl et al., 2017). Egg washing
Egg washing is practiced routinely in many countries such as Australia and the United States but is generally prohibited for Class A eggs in the European Union. Inappropriate egg washing practices may be ineffective or actually make the situation worse. (Messes, 2011; Sexton, 2017). Egg washing equipment has improved in recent years to the extent that some of the earlier concerns have been addressed satisfactorily. If conducted correctly, egg washing reduces the microbial load on eggshells. Sexton (2017) outlines the various stages of the egg washing process and the critical aspects of each of these stages. Things to be considered are cleaning time, the temperature of the wash water (to prevent water being sucked into the egg contents), agitation, cleaning chemicals used as well as their concentration and application, and the use of a sanitizer. Eggs need to be rinsed and dried. Egg washing equipment needs to be decontaminated by cleaning and sanitizing after each production period. The entire washing process needs to be monitored, verified and validated.
Safe food handling practices

In addition to precautionary practices at the level of the farm and processing facility, safe food handling practices in commercial food preparation and in the home are important. Egg industry organisations in many countries provide information and guidelines to reduce the risk of foodborne illness (Australian Egg Corporation Limited, 2010). For example, eggs sold for retain and catering purposes should not be cracked or dirty; eggs should be stamped to facilitate trace-back; eggs should be stored at a temperature of 15°C or less, kept in their original packaging and stored away from cooked or ready to eat foods. When handling eggs, avoid contact between the egg shell and egg contents, and wash and sanitise surfaces that have been in contact with eggs. Particular care needs to be taken when preparing foods with uncooked or only partially cooked eggs.

In many countries, government and industry bodies provide guidelines about safe food preparation when using eggs. In Australia, the national industry body, Australian Eggs, have prepared a range of educational tools including posters to assist those in the food preparation industries to minimise risk of foodborne illness associated with eggs (Australian Eggs, 2019) and similar tools are produced in other countries such as the United States (U.S Food and Drug Administration, 2016). Education of the general public in the safe use of eggs and egg products can further reduce the incidence of foodborne disease. Australian Eggs has also produced educational tools aimed at the public to facilitate safe handling processes in the home.

Conclusion

Safe consumption of eggs and egg products depends on precautions taken at all stages of the egg production chain “from farm to fork”.

References


Eggshell Structure, Mineralization and Quality: an Update

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Abstract

The avian eggshell is a thin mineral layer (about 350 µm thick in chicken) that protects the egg contents against mechanical impacts, dehydration and microorganism contamination. Its porous structure allows gaseous exchange and supplies calcium needed for embryonic development. The eggshell is constituted by columnar calcite crystal units that incorporate a substantial amount of organic matter (3.5%). Eggshell formation is a highly controlled and rapid mineralization process occurring while the egg remains in the uterus during the night. Shell mineralization requires a large and continuous supply of calcium that comes in part from the diet and in part from the skeleton (medullary bone). Medullary bone serves as a calcium reservoir for eggshell calcification during the night and its resorption and formation synchronized with eggshell formation. The composition of the uterine fluid changes at each stage of eggshell formation (initiation, linear growth, termination), with the sequential expression of specific proteins that actively change the mineral properties. It ends at the vertical crystal layer, which is a thin layer of small and thin calcite crystals arranged vertically.

Eggshell Structure and composition

The avian eggshell is a thin mineral layer (about 350 µm thick in chicken) that protects the egg contents against mechanical impacts, dehydration and microorganism contamination (Nys et al., 1999; Hincke et al., 2012). It is pierced by numerous pores that allow the gaseous exchange associated with embryonic respiration. It also supplies the calcium needed for skeletal development.

The eggshell is composed of the eggshell membranes, the eggshell mineral and the cuticle that coats the outer eggshell surface (Fig. 1). The eggshell membranes are a meshwork of highly cross-linked protein fibres made of collagen (mainly type X), glycoproteins and cysteine-rich eggshell membrane proteins (Cordeiro and Hincke, 2016). They also contain soluble proteins with antimicrobial properties (i.e. lysozyme, ovotransferrin and ovocacyxin-36). There is a thinner inner membrane located upon the limiting membrane that surrounds the egg white, and a thicker outer membrane attached to the tips of the mammillary cones (inner shell mineral). Interestingly, the membrane properties changes during incubation, which increases oxygen permeability. The mammillary cones arise from organic aggregates rich in sulfated proteoglycans (mammillary knobs) that are distributed pseudo periodically on the surface of the outer shell membrane. These structures have a strong calcium binding capacity and act as nucleation sites for calcite crystal formation as demonstrated also in vitro (Fernandez et al., 2004). From the mammillary knobs, cone shaped mineral structures emerge and meet at the base of the palisade layer that is constituted by columnar units arranged perpendicular to the eggshell surface. The palisade layer comprises most of the thickness of the eggshell mineral part and has the greatest contribution to eggshell mechanical properties. It ends at the vertical crystal layer, which is a thin layer of small and thin calcite crystals arranged vertically. Finally, the outer surface of the eggshell is coated by the cuticle, a very thin (a few microns thick) organic layer that protects the pores to control shell permeability and prevent bacterial ingress through the shell (De Reu et al., 2006; Bain et al., 2013; Mühle et al., 2015). Additionally, the cuticle contains proteins (lysozyme C, ovotransferrin, ovocacyxin-32) and lipids with potent antimicrobial activity (Rose-Martel et al., 2012). Thus, the cuticle, if present, is an effective barrier against bacteria penetration and is of great importance for the food safety of eggs.

Keywords: eggshell quality, calcium metabolism, medullary bone, cuticle, salmonella.

Eggshell Structure, Mineralization and Quality: an Update

During the daily egg laying cycle, there are also notable changes in hen physiology in order to deliver large amounts of calcium and carbonate ions across the uterine tissues (Nys and Le Roy, 2018). When hens reach sexual maturity, at about 16 weeks of age, oestrogen levels increase and the eggviduct starts growing very rapidly. Two weeks later, they lay their first egg. Eggshell formation and mineralization is a highly calcium-demanding process. Hens need to mobilize more than 2 g of calcium daily, equivalent to 10% of their total body calcium (Nys and Guyot, 2011). In general, calcium comes in part from the diet and in part from the skeleton; the relative contribution varies depending on the availability of dietary calcium (Taylor and Moore, 1954). To obtain an adequate supply of calcium, a stimulated production of vitamin D leads to an increase in calcium absorption by intestinal and uterine tissues. Additionally, hens develop a new type of bone within the narrow cavities of their long bones (medullary bone) that is metabolically active and can be more easily resorbed to release calcium.

Medullary bone forms as a calcium reservoir for eggshell calcification during the night when hens are not eating and the intestinal calcium absorption is exhausted. The formation of medullary bone starts about 15 days before laying the first egg when the hen reaches sexual maturity. The increasing oestrogen levels stimulate osteoblast function to switch from producing cortical bone to the forming of medullary bone. During this period, skeletal weight increases by 20% (Whitehead, 2004).

During the daily egg laying cycle, there are also notable changes in hen physiology in order to deliver large amounts of calcium and carbonate ions across the uterine tissues (Nys and Le Roy, 2018). In the afternoon, just before eggshell formation starts, hens develop a specific appetite for calcium. During eggshell formation, the production of vitamin D is stimulated, which increases calcium absorption by intestinal tissues. Moreover, medullary bone resorption and subsequent formation is stimulated in synchrony with eggshell formation. Thus, there is an intense osteocalcin-mediated resorption of medullary bone followed by intense osteoblastic activity to form new medullary bone before the next cycle of eggshell formation (Van de Velde, 1984; 1985). Both processes are regulated by PTH hormone and vitamin D secretion to maintain constant blood calcium levels. Additionally, during eggshell formation...
Avian egg formation is a highly controlled process in which the forming egg passes through specific segments of the oviduct to sequentially acquire its specific components. The shell biomineralization process can be divided into three main stages (initiation, linear growth and termination) (Nys et al., 1999). At the initiation stage (approximately 6 hours post-ovulation (p.o.)), the forming egg briefly resides in the isthmus, where organic components (proteoglycans) are secreted and accumulate on the outer surfaces of the eggshell membranes. Rapid eggshell mineralization occurs in the uterus (the shell gland) during the linear growth stage (between 7 to 22 hrs p.o.), the eggshell is rapidly mineralized at a constant rate until shell deposition is actively arrested at about 22hrs p.o., and the cuticle is deposited, about two hours before oviposition (expulsion). During the entire process, the mineralizing egg is bathed in the uterine fluid, an environment that contains a high concentration of organic matrix (proteins and proteoglycans) and inorganic precursors (calcium and carbonate ions). This milieu is highly supersaturated with respect to all calcium carbonate polymorphs (aragonite, vaterite and calcite) (Nys et al., 1991; Rodriguez-Navarro et al., 2015).

Eggshell mineralization begins with the deposition of massive mineral deposits of amorphous calcium carbonate (ACC) on the mammillary cores, which progressively dissolves and gives way to calcite crystals (Rodriguez-Navarro et al., 2015). ACC and calcite crystals form by aggregation of nanoparticles, an efficient growth mechanism which can explain the rapid mineralization of the eggshell. Moreover, its formation through dissolution of an intermediate amorphous mineral (ACC) allows calcium carbonate mineralization to take place in the uterine fluid and contributes in addition to Ca secretion to a continuous supply of calcium to sustain fast and continuous growth of calcite crystals during the linear phase of eggshell formation. As in other controlled biomineralization processes, the organic matrix plays a key role in the modulation of shell mineralization. The composition of the uterine fluid changes at each stage with the expression of specific proteins (i.e. ovobain, lysozyme, ovotransferrin, ovocleidin-17 and -116) that became concentrated in specific regions of the eggshell (i.e., mammillary cores), indicating that they are involved in the nucleation and stabilization of calcium carbonate mineral (ACC, calcite) and in the regulation of eggshell mineralization (Gautron et al., 1997; Nys et al., 1997; Marie et al., 2015).

Eggshell quality

Cracked and damaged eggs account for about 6-8% of total egg production; their downgrading causes substantial economic losses to the egg producing industry (Hamilton et al. 1979). Moreover, eggshell quality deteriorates with increasing hen age; this downgraded percentage can rise to 20-30% of eggs at 65-70 weeks of age and is one of the main reasons to limit the production cycle to one year (Nys et al., 1999; Travel et al., 2011; Bain et al., 2016). The gradual decline in eggshell properties (i.e., breaking strength) with hen age is partly due to the fact that the amount of shell mineral deposited is kept nearly constant during the production cycle (about 6 g) while the egg size slightly increases with hen age (from 60 to 67 g) though recently this change with age has been reduced. Still, the shell weight percentage and eggshell thickness tend to decrease as the hen ages. However, there are notable changes with age in the eggshell ultra- and microstructure characteristics (i.e., decreased mammillary density with poor membrane attachment, increased core size of calcite crystals that are the main cause of eggshell quality and integrity to diminish in old hens (Rodriguez-Navarro et al., 2002; Robert et al., 2013; Park and Sohn 2018). Now that the industry is aiming to extend the egg-laying period until hens age reaches 100 weeks and could achieve a production of 900 eggs per hen, there is renewed interest in finding solutions to maintain hen laying performance and egg quality for longer periods of time (Bain et al., 2016; Nys, 2017). Poor eggshell quality is also a major concern for food safety, as eggs with a damaged eggshell are more easily contaminated with bacteria (i.e., Salmonella) and pose an important risk to consumers (Hamilton et al., 1979). The eggshell characteristics related to quality are greatly influenced by a wide array of factors including hen age, genetics, nutrition as well as environmental factors (hunting, lighting program) (Dunn et al., 2009; Rodriguez-Navarro et al., 2005; Nys and Guyo, 2011; Nys et al., 2017). A complete assessment of eggshell quality should include not only its thickness and/or mechanical properties but also an examination of eggshell structure, eggshell membranes and the cuticle. New tools have been developed in the last decades to assess eggshell properties (i.e., dynamic stiffness) that can better predict eggshell breakage in the field (Bain et al., 2006). Other relevant eggshell properties are the ultra- and microstructure characteristics (mammillary density, crystal size) that can be improved by nutrition (i.e., organic matrix) that can better predict eggshell breakage in the field (Bain et al., 2006). Other relevant eggshell properties are the ultra- and microstructure characteristics (mammillary density, crystal size) that can be improved by nutrition (i.e., organic matrix) that can better predict eggshell breakage in the field (Bain et al., 2006). Other relevant eggshell properties are the ultra- and microstructure characteristics (mammillary density, crystal size) that can be improved by nutrition (i.e., organic matrix) that can better predict eggshell breakage in the field (Bain et al., 2006).

Eggshell formation

Eggshell formation is described as the high controlled biomineralization regulated by specific organic components that are secreted by the uterus tissue. Eggshell formation is also a highly demanding process that require specialized physiological adaptation of the hen before egg laying begins. Maintaining egg production and particularly eggshell quality through extended egg laying cycles (until hens are 100 weeks old) is challenging but is currently achieved by genetic assisted selection programs aimed to improve most relevant eggshell quality traits. Nevertheless, hen nutrition needs to be optimized to achieve and maintain high eggshell quality in old hens.

REFERENCES


Keel bone damage in laying hens

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Abstract
Keel bone damage is one of the largest problems within the commercial laying hen industry where the majority of hens manifest some level of damage by end of lay. A specific type of damage, fractures to the keel bone, represent both a welfare and production issue. Beyond the presence of gross anatomical disfigurement, hens with fractures have altered behavior suggesting the experience of pain. Regarding productivity, hens with fractures have reduced egg production and are likely increasing feed consumption to compensate for the healing process following injury. Keel bone damage is believed to be a product of relatively high egg production leaving bones brittle and prone to damage by collision, though recent evidence suggests a more complex picture where pathological fractures or insufficient bone development may also be factors. Ongoing research efforts are working to identify how fractures can best be identified, the most plausible mechanisms to explain the cause of fractures, and effective interventions. To coordinate these efforts and ensure stakeholders remain informed as to the latest developments, an ongoing EU-COST Action, KeelBoneDamageNet (www.keelbonedamage.eu), is currently running until 2021.

Introduction
The keel bone of laying hens and the high frequency of fractures seen in this bone within commercial systems represent one of the greatest welfare problems facing the industry as suggested by the UK’s Farm Animal Welfare Committee (FAWC, 2010, 2013). Beyond the obvious welfare issue of gross skeletal deformities, concern stems from the likely associated pain and suffering indicated by altered behaviour in birds with fractures that was reduced with anti-inflammatory agents and analgesics (Naur et al., 2012b; c, 2015) but unexpectedly this has been associated with an increased prevalence of keel bone fractures in laying hens. Bone fractures are acutely painful in mammals, but the effect of fractures on bird welfare is unclear. We recently reported that keel bone fractures have an effect on bird mobility. One possible explanation for this is that flying becomes mechanically impaired. However it is also possible that if birds have a capacity to feel pain, then ongoing pain resulting from the fracture could contribute to decreased mobility. The aim was to provide proof of concept that administration of appropriate analgesic drugs improves mobility in birds with keel fracture; thereby contributing to the debate about the capacity of birds to experience pain and whether fractures are associated with pain in laying hens. In hens with keel fractures, butorphanol decreased the latency to land from perches compared with latencies recorded for these hens following saline (mean (SEM). The keel is a bone extending from the sternum (King, 1975) and fractures can be defined as breaks that will typically manifest as a callus around the fracture site though may also involve sharp, unnatural deviations or bending (Casey-Trott et al., 2015). The problem is widespread with similar levels reported in various countries (UK (Wilkins et al., 2011) the presence of keel fractures was determined and the tibia, humerus and keel bones dissected for measurement of breaking strength. For each house, variations in internal design and perching provision were categorised and the effective heights of the differing structures recorded. All systems were associated with alarmingly high levels of keel damage although variation in mean prevalence between systems was evident with flocks housed in furnished cages having the lowest prevalence (36 per cent; Holland (Rodenburg et al., 2008); Belgium (Heerkens et al., 2015); Denmark (Riber and Hinrichsen, 2016); and Canada (Petrik et al., 2015)). The issue appears to extend across all types of housing systems (Wilkins et al., 2015). A problem that has been consistently reported to occur across all housing systems is a reduction in egg production following keel bone fractures. This is especially true in those cases where the hen has been heavily producing. However, the degree to which egg production is affected is variable and dependent on the extent of the fracture. It is possible that a more severe fracture will result in a lower egg production than a less severe fracture, but in general it has been suggested that egg production is reduced to some degree when a keel bone fracture occurs. However, it is currently unknown whether the degree of egg production reduction is influenced by the extent of the fracture. The causes of keel bone fracture The causes of keel bone fractures are multifactorial, involving a host of environmental, nutritional, and genetic factors that result in varying manifestations of frequency and severity of keel bone fracture across age, housing system, and management styles. Despite the complicated mixture of factors, it is generally believed the principal cause is a relatively high and extended period of egg production and shell formation. This leaves the bone weak, brittle, and thus prone to fracture (Whitehead and Fleming, 2004; Whitehead, 2000; Whitehead, 2004). Supporting this theory, Eusemann et al. (Eusemann et al., 2018a) found reduced fractures in a low producing brown laying line. Additionally, the experience of pain has also been shown to be a contributing factor to increased egg production following keel bone fractures. This is evidenced by the decreased latency to land observed following butorphanol treatment (Casey-Trott et al., 2015). The causes of keel bone fractures are multifactorial, involving a host of environmental, nutritional, and genetic factors that result in varying manifestations of frequency and severity of keel bone fracture across age, housing system, and management styles. 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Influence of keel bone fractures on hen productivity

Results for altered egg production in individual birds were previously reported by Nasr et al. (2012b, 2013) egg weight and feed and water consumption in individual laying hens. A total of 165 Lohmann brown laying hens were obtained from a commercial farm that consisted of 105 with keel fractures and 60 without keel fractures. 2. After a 4-d period of acclimatisation, hens were individually housed and provided with ad libitum food and water for a 24-h period. The number of eggs laid, egg weight, feed and water consumption during this period were recorded. Keel bone strength was also assessed. 3. Hens free from keel fractures laid more eggs (91.7%) vs. 84.9%. Although this was assessed in non-commercial conditions, it is generally accepted that commercial systems have a weakened state, it is thought that she is more susceptible to fractures resulting from collisions and crashes (Wilkins et al., 2011). Petri et al., 2015) Canada, that housed hens in cages (n = 9). Wilkins et al. (2011) the presence of keel fractures was determined and theibia, humerus and keel bones dissected for measurement of breaking strength. For each bone, variations in internal design and perching provision were categorised and the effective heights of the differing structures recorded. All systems were associated with alarmingly high levels of keel damage although variation in mean prevalence between systems was evident with flocks housed in furnished cages having the lowest prevalence (36 per cent reasoned the variation in fractures observed between systems was likely due to perches causing more high energy collisions, a view supported by increased fracture occurrence and severity seen in systems with more and higher items available for perching. As already mentioned, fracture also appear in non-cage systems where high energy collisions are less common though have been shown to occur (Baker et al., submitted to AAIB). Although extended egg production is believed to be a critical if not primary factor, recent considerations of alternative explanations, such as existing pathological conditions (Harlander-Matuschek et al., 2015), also need to be considered.

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Influence of housing system and design on bone strength and keel bone fractures in laying hens


Laying hen nutrition: reaching the full potential of the bird

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Abstract

The main evolution in egg production is the extension of the production period due to genetic improvement in persistency and egg quality with hen’s age. Hen nutrition remains a crucial parameter for sustainability of the egg production system to ensure high performance. The limit of laying period extension is still determined by controlling, through nutrition, the quality of the final product and avoiding any deterioration in the hen health. The main problems to be solved are the control of the egg weight, quality of internal egg and eggshell, to avoid metabolic disturbance (liver and bone) feather pecking and to deal with the heterogeneity of hen performance in the flock. Precise knowledge is available on hen nutrition but the difficulties result from the diversity of factors and their interactions. Future development in modelling might help in integrating such complexity throughout all the rearing and production periods. The rearing period is important for the future production of hens. Any deviation from optimia might have long term effects. There is also a need for more information to be available throughout different seasons and equipment’s especially in alternative systems. In terms of sustainability, nutrition remains a crucial factor because of the similarities in hen genetic background used throughout the world and because the differential cost of production between different areas mainly results from the availability and cost of feedstuffs. This review underlines the main nutritional factors affecting egg quality and proposes strategies to keep egg quality in long laying cycle.

Introduction

The priority of breeding companies is currently to increase persistency in lay and maintain egg quality in older hens (Bain et al., 2016). Selection of egg persistency, combined with improvement in eggshell and internal egg quality in hens at older ages, has enabled egg production to be extended to more than 85 weeks of age (Dunn, 2013, Pottguetter, 2015). The aim is currently to extend the laying cycle of commercial flocks to 100 weeks which corresponds to an exportation of 25 kg of eggs (about 14 folds the hen’s body weight) and more than 1kg of calcium (3 kg of eggshell). This challenges the metabolism and homeostasis of the hen. Lower shell quality is currently the main reason to terminate a layer flock, rather than the persistency of egg production. Internal quality (Haugh unit and strength of the vitelline membrane) is also one of the limiting factors. Hen nutrition is therefore crucial, in optimising the excellent genetic potential of modern hens in terms of productivity and high egg quality, but also for avoiding any health problems in aged birds such as bone demineralisation or fatty liver syndrome. This review aims to underline some nutritional factors which might help to extend the laying period and prevent any unexpected metabolic pathologies. More detailed information is available in previous reviews (Bouvarel and Nys, 2013; Nys 2017a, b).

Feeding the pullet and pre-laying phase

Nutrition of the pullets influences the growth curve, live weight and body composition at sexual maturity, parameters which impact egg production and quality. It is well established that the body weight of hens at the onset of lay is highly correlated with initial egg weight and to a lesser extend with that of mature hens therefore influencing the egg mass (Bouwarel and Nys, 2013; Nys, 2017a). This is partly explained by the competition in energy requirement for the late growth of the pullets and the initiation of egg production, combined with the limited capacity for feed intake in young modern hens. It is important during the intermediary period (9-16 weeks) to reinforce the development of the gastrointestinal tract and the hen’s capacity to increase feed intake. This favours during the transition phase (16-20 weeks) the protein, energy and calcium supply needed for the initiation of egg production. The initial growth of internal organs, muscles and the active development of the skeleton until about 8 weeks are heavily dependent on protein and amino acid intake and calcium, phosphorus supply. Keshavarz and Jackson (1992) demonstrated an increase of 0.32g of bone weight per supplemental protein point when supplying 11 to 20 % dietary proteins throughout the pullet phase. During the first period (<8 weeks) optimal levels of energy (12.33-11.7 kJ AMEn/kg, protein (20-18%), digestible amino acids (0.48-0.4 methionine; 1.2-1.0 lysine) and minerals (1% calcium) should be provided. The supply of phosphorus should be high enough (>4 NPP) during the starting and growing period to ensure bone growth. The switch to developer diet should be carried out when targeted body weight is reached for a genetic line, rather than at a fixed age of the pullets as recommended by breeding companies. During the intermediary phase (9-16 weeks) the protein and mineral requirement are lower and the ratio of protein to energy should be optimized to avoid fattening of the birds. There is evidence that the relationship of body development at 12 to 13 weeks of age with egg weight will be stronger than that at 18 weeks because nearly 95 % of the body frame is developed at this age (Thiele, 2012). It will be more difficult to compensate for a lower body size after 12 weeks by increasing energy feed density because overconsumption of feed will favour the development of fat and such change in body composition has been associated with a lower egg weight from 20 to 64 weeks of age (Cheng et al., 1991). The developer diet should contain 5 to 6 % crude fibre to stimulate the development of the intestinal tract and this period will be used for training birds to eat more as modern hens show difficulties in consuming enough feed at the onset of lay (Pottguetter, 2015). The particle size is also stimulating feed intake and digestive capacity. During the initial period (<45 days of age) small particle size (screen size <8 mm) is more suitable to beak size and favours feed intake (Frikha et al. 2011) but during the intermediary period, use of whole cereals or crumble diet combined with frequent and
limited distribution of feed promotes feed intake and develops digestive tract capacity (Guzman et al., 2015). Bone growth is limited when total phosphorus was increased from 0.18 to 0.32 % NPP.

Energy.

During the laying period, and in particular in extended production cycles, the challenge will be to supply a diet adjusted to energy and protein requirements to optimize egg output, and in particular egg weight, without increasing body weight and fatness, avoiding hepatic steatosis and bone demineralization and maintaining good feathering by limiting feather pecking. Feed composition should be adjusted continuously to daily feed intake for controlling egg weight or quality. In older flocks, there is increased heterogeneity of individual birds. In this case, nutritionists should adjust the feed composition to keep in lay the most productive hens. The energy requirement of laying hens is due to maintenance and egg production. It is influenced by body weight, egg production and to a lesser extent by environmental temperature, activity and hen feathering. It has been estimated by Joly (2005) in brown egg layers: Daily Energy requirement (kcal) = 130% body weight (kg)0.75 + 1.8 Egg mass (g) + 6 daily weight gain (g/day) temperature at 22°C, *For white leghorns: 137*. The energy cost per gram of egg is therefore estimated to be 1.8 kcal. At temperatures lower than 22°C, it increases by 2 kcal per degree and kg body weight and decreases by about 1 kcal between 24 and 28°C (Joly, 2005).

In alternative systems, the requirement for maintenance is higher due to locomotor activity by about 1 kcal/d. Feed consumption can be increased by increasing the horizontal movement of the flock. Feed intake and egg weight is frequently observed in flocks of old hens and increases the maintenance energy requirement. (Peguri and Coen, 1993).

Control of feed consumption

Hens have the ability to adjust their feed consumption based on dietary energy concentration (Thieule, 2012). Energy concentration can thereby be increased by feeding layers located between 1.18 and 1.5 mm (Bouvarel and Nys, 2013) and increasing Dif- ference in consumption (%) = 1.452. 0.685 x difference ME (%) = R2 0.875. This adjustment is linear but partial as the hens cannot compensate a high dilution of the diet or, conversely, decrease their feed intake to adapt to high density diet. It is also possible to increase the daily energy intake by enriching the diet with fat (1.3% /100 kcal; Valkomen et al., 2008; Van Krimpen et al., 2009; Perez-Bonilla et al., 2012). This option is used for supplying enough energy at the onset of egg production or for increasing the egg weight in brown egg laying hens. In Lgehorns hens, this capacity of adjusting feed intake to energy dilution is not observed (de Persio et al., 2015) as a linear reduction in egg production and weights are observed when diets are diluted by 5 or 10%. The control of feed consumption of laying hens firstly depends on energy requirement but is also influenced by their ability to recognize particles in the diet. Laying hens clearly adapt their feed intake according to the relative size of the particles in relation to the beak. By using feeds presented as crumbs (72% of particles > 1.18 mm) Portella et al. (1988) demonstrated an immediate effect of feed consumption. Although the consumption of feed intake is low in crumbs, the feeding of laying hens in fibres. The hens have the ability to select diet components when particles such as grains are present. Such use of whole cereal meals produced on the farm can reduce feed costs. Sequential, loose-mix and choice feeding offer birds the opportunity to select different diets in short term and to match nutrient supply to individual requirements during the daily changes induced by the temporal sequence of the egg formation (Molnar et al., 2010). Production performance of birds are generally not affected by the type or form of the grain used in free choice feeding (Forbes and Covasa, 1995). Keshavzara (1998a, b) evaluated sequential supply of diets during the day (positive 16% CP, negative control 13% CP, two sequential diets combining 13% and 16% CP fractions) and concluded that optimum performance may be expected as long as the daily protein intake is adequate and suitable for the daily needs of laying hens. Whole grains supplying mainly energy can also be provided along with a complete feed concentrating the supply of proteins, minerals and vitamins as a mixture, or sequentially during the day. Blair et al. (1973) reported that the sequential supply of cereals (wheat, barley and corn) in the morning and a balancer diet (dried in protein, minerals and vitamins to provide the daily requirement of hens) in the afternoon, did not result in a deterioration in egg weight. Umar-Faruk et al. (2010) showed that the use of a cut feed (50% of particles < 3 mm) resulted in a higher feed intake and therefore egg production.

Phosphorus is an essential nutrient for numerous metabolic functions in laying hens, from the cell level to the whole organism. P deficiency induces many metabolic disorders resulting in deterioration of bone status, appetite and reproductive functions. The major part of body phosphorus is present in bone and a particular form of bone, the medullary bone, develops in hens at sexual maturity. It is used as a calcium reservoir throughout the laying period to compensate for any inadequate dietary Ca supply for shell mineralization mainly during the first months of the laying period (Carberry and Stone, 1977). The maximum contribution of bone Ca can reach 40% of the eggshell (about 1g) and concomitantly will liberate 0.42 g of phosphorus which will be mainly excreted in the urine. The intense mobilization of bone associated with egg production can lead to osteoporosis and create a risk of bone breakage particularly in aged hens (Whitehead, 2004). The effects of dietary levels of phosphorus with a frequency used range (0.35 to 0.75 % total phosphorus) on egg quality (egg weight, eggshell quality) are rather limited. Egg production and hen mortality are more sensitive to the supply of phosphorus. The main question for poultry nutritionists is to know how far it is possible to reduce the supplementation of inorganic phosphorus in hens to limit its negative environmental impact and its cost. Since 1997, the European Community has been working on the reduction of the contribution of phosphates to animal feed. In the future, it is possible to replace inorganic phosphates by phytase. Phytase phosphorus is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase.

Feed management

When complete diets are used, feed intake is mainly controlled by the hens’ energy requirement and feed presentation, therefore the bird’s nutritional need to be balanced with the other nutritional requirements and then the egg shell formation. The hens have the ability to select diet components when particles such as grains are present. Such use of whole cereal meals produced on the farm can reduce feed costs. Sequential, loose-mix and choice feeding offer birds the opportunity to select different diets in short term and to match nutrient supply to individual requirements during the daily changes induced by the temporal sequence of the egg formation (Molnar et al., 2010). Production performance of birds are generally not affected by the type or form of the grain used in free choice feeding (Forbes and Covasa, 1995). Keshavzara (1998a, b) evaluated sequential supply of diets during the day (positive 16% CP, negative control 13% CP, two sequential diets combining 13% and 16% CP fractions) and concluded that optimum performance may be expected as long as the daily protein intake is adequate and suitable for the daily needs of laying hens. Whole grains supplying mainly energy can also be provided along with a complete feed concentrating the supply of proteins, minerals and vitamins as a mixture, or sequentially during the day. Blair et al. (1973) reported that the sequential supply of cereals (wheat, barley and corn) in the morning and a balancer diet (dried in protein, minerals and vitamins to provide the daily requirement of hens) in the afternoon, did not result in a deterioration in egg weight. Umar-Faruk et al. (2010) showed that the use of a cut feed (50% of particles < 3 mm) resulted in a higher feed intake and therefore egg production. Furthermore, the effects of dietary levels of phosphorus with a frequency used range (0.35 to 0.75 % total phosphorus) on egg quality (egg weight, eggshell quality) are rather limited. Egg production and hen mortality are more sensitive to the supply of phosphorus. The main question for poultry nutritionists is to know how far it is possible to reduce the supplementation of inorganic phosphorus in hens to limit its negative environmental impact and its cost. Since 1997, the European Community has been working on the reduction of the contribution of phosphates to animal feed. In the future, it is possible to replace inorganic phosphates by phytase. Phytase phosphorus is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase. It is recommended to introduce two third of Ca phosphorus and it is considered that more than 70 % of PP is hydrolysed by recent batches of commercially available phytase.
termination of egg production for a flock. Calcium is the key nutrient influencing shell strength (Classen and Scott, 1982; Hartel, 1990). Calcium requirements have been established at 3.5 to 4.5% during the laying period. Hens export 2.2 g calcium per egg and at least 4 g per day when taking into account mean calcium retention (Nys, 1999). A limited supply of calcium reduces shell strength and egg production, and increases mortality (Hartel, 1990; Keshavarz, 1998 a,b; Kebeab et al, 2009). Phase feeding throughout the laying year (3.5, then 4.5, and finally 5.5% calcium in the feed) might slightly limit the deterioration in shell strength and in bone reserve with increasing hen age (Valkonen et al. 2009). The selection for increased egg numbers is associated with a daily production of an egg which is laid very early. Consequently, it might be difficult to turn hens to lay at the required age when the supply of calcium required for eggshell formation which mainly occurs during the night. All nutritional management (midnight feeding; alternative feeding with various diets; Ca particles) favouring synchronization of dietary calcium intake with the period of shell formation will reinforce shell strength (Nys, 1999). European legislation regarding bird welfare, however, limits application of lighting programs and has banned nocturnal flashes of light. Hens show, however, remarkable capacity of their calcium metabolism for supplying Ca for the eggshell (Bar, 2008; 2009; Nys and Leroy, 2018). First, hens express a specific appetite for calcium (Mongin and Sauveur, 1979; Wilkinson et al., 2011) a few hours before calcification takes place, favouring storage of feed including calcium in the crop and in acid secretion induced by dilatation of this organ (Lee et al., 1988). This favours storage and solubilisation of the dietary calcium throughout the night, especially when coarse particles are available to the hens. The provision of coarse calcium particles limits the hen’s need to mobilise calcium and phosphorus from the bone reserve, decreases bone phosphorus elimination (Whitehead, 2004; Fleming et al. 2008) and reinforces bone strength (Guinotte and Nys, 1993, Fleming et al, 1998). Particulate calcium sources reveal different physico-chemical properties and composition in Ca and trace elements (Guinotte and Nys, 1991) so it is important to determine the composition of a new source and trace elements composition of the Ca source. Calcium has limited influence on shell quality in contrast to the size of particles. Particles have to be greater than 0.8 mm in order to be retained in the gizzard (Rao and Rolando, 1990). In addition, hens fed mixed particle sizes have greater feed intake than those fed a 100% ground calcium core (large particle size). Large particle size may result in a higher intake of feed to reach a DEB of 360 mEq/kg compared to those fed a DEB of 240 mEq/kg. In older hens (45-55 wks) Gezen et al., (2009) found that the use of Ca particles helps counteract situations unfavourable to shell quality (old hens, heat stress, low dietary Ca intake and convincingly favours bone strength. It is recommended to increase the proportion of large particles relative to ground material when the hens are older.

### Electrolyte balance

Hens acid-base metabolism corresponding to the homeostasis of pH in cells and internal fluid is strongly influenced by the eggshell formation because of exportation of large amounts of carbonate and associated production of H⁺ which are reabsorbed by the uterus influenced by electroneutrality conditions. As an addition, the large exportation of carbonate of Mg²⁺ and anions (Cl⁻, H₂PO₄⁻, HPO₄²⁻, SO₄²⁻). The lower incorporation of sodium in the composition of eggshell has limited influence on shell quality in contrast to the size of particles. Particles have to be greater than 0.8 mm in order to be retained in the gizzard (Rao and Rolando, 1990). In addition, hens fed mixed particle sizes have greater feed intake than those fed a 100% ground calcium core (large particle size). Large particle size may result in a higher intake of feed to reach a DEB of 360 mEq/kg compared to those fed a DEB of 240 mEq/kg. In older hens (45-55 wks) Gezen et al., (2009) found that the use of Ca particles helps counteract situations unfavourable to shell quality (old hens, heat stress, low dietary Ca intake and convincingly favours bone strength. It is recommended to increase the proportion of large particles relative to ground material when the hens are older.

### Trace elements

The novel legislation tends to decrease the dietary level of trace elements in animal nutrition to avoid negative impact on the environment affects the formation of eggshell. The essential trace elements (Fe, Zn, Cu, I, Se) are involved in the biochemical and mechanical properties of eggshell membranes (Chowdhury, 1998) resulting in egg shape abnormalities. Eggshells from hens fed Mn deficient diets (<7 mg) showed lower eggshell thickness, translucent areas due to alterations in eggshell ultrastructure, and a reduction in the size of particles limits the hen’s need to mobilise calcium and phosphorus from the bone reserve, decreases bone phosphorus elimination (Whitehead, 2004; Fleming et al. 2008) and reinforces bone strength (Guinotte and Nys, 1993, Fleming et al, 1998). Particulate calcium sources reveal different physico-chemical properties and composition in Ca and trace elements (Guinotte and Nys, 1991) so it is important to determine the composition of a new source and trace elements composition of the Ca source. Calcium has limited influence on shell quality in contrast to the size of particles. Particles have to be greater than 0.8 mm in order to be retained in the gizzard (Rao and Rolando, 1990). In addition, hens fed mixed particle sizes have greater feed intake than those fed a 100% ground calcium core (large particle size). Large particle size may result in a higher intake of feed to reach a DEB of 360 mEq/kg compared to those fed a DEB of 240 mEq/kg. In older hens (45-55 wks) Gezen et al., (2009) found that the use of Ca particles helps counteract situations unfavourable to shell quality (old hens, heat stress, low dietary Ca intake and convincingly favours bone strength. It is recommended to increase the proportion of large particles relative to ground material when the hens are older.

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and therefore it is recommended to provide dietary feedstuffs (about 1000 mg/kg). Osteoporosis results from loss of structural calcium particles limits the hen’s needs to mobilise calcium and phosphorus from the bone reserve, decreases bone phosphorus concentration, and therefore, increases the risk of bone fracture. The incidence of osteoporosis is high in Europe, as indicated by the percentage of hens showing bone fracture at the end of the laying period (up to 30%, Gregory and Wilkins, 1989). Preventing osteoporosis

Feather pecking and cannibalism are important welfare issues (Blokhuis et al, 2007) the impact of which might be increased by the extension of the laying period as feather loss is frequently observed in flocks of old hens. Feather coverage corresponded to 50% normal, +7 g at temperature of 23.9°C (Peguri and Coon, 1991). Feather pecking can require additional nutrient content in complete feed. Preventing feather pecking

Preventing osteoporosis

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**XVIII European Symposium on the Quality of Eggs and Egg Products**

**Oral Presentations**
With the availability of the 60K Affymetrix® Axiom® high-density (HD) single nucleotide polymorphism (SNP) chip, genomic selection has been implemented in broiler and layer chicken. However, the cost of such HD SNP chip is still too high to genotype all selection candidates. A solution is to develop low density SNP chip, at a lower price, and to impute all missing markers before doing a genomic evaluation. However, the impact of the direct use of low density SNP chip on genomic evaluation accuracy is not well documented. In this perspective, the interest of using or not imputation in genomic selection was studied in a pure layer line. An equidistant methodology was used to design low density SNP chips. Egg weight and egg shell strength were evaluated with ssGBLUP methodology. The impact of imputation errors or the absence of imputation on the ranking of the selection candidates was assessed with a genomic evaluation based on ancestry. To do so, genomic estimated breeding values (GEBV), with imputed or not imputed genotyping from the low density SNP chip, were compared to the GEBV obtained with the HD SNP chip. The accuracy of GEBV was also investigated by considering as reference the GEBV estimated on offspring. With imputed low density genotyping, the ranking was slightly more correlated to that with HD genotyping than that with low density genotyping. However, Spearman correlations were above 0.95 with more than 5K SNPs. Thus the reordering of the breeders can also be a solution but the decreasing trend observed for GEBV accuracy with very low density seemed to depend on the trait evaluated.

Keywords: Genomic selection, Low density chip, Imputation, Evaluation accuracy

Guinea Fowl Eggshell Structural Organization and Particular Organic Matrix Protein Patterns to Decipher its Exceptional Mechanical Properties

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Guinea fowl (Numididaegeaisis) presents a highly resistant eggshell compared to the other birds. We will described in this study the particular ultrastructure of the Guinea fowl shell that confers exceptional mechanical properties and how changes in organic matrix components controls the development of this structure. The inner part of the shell is similar to other birds, but an additional microstructure shift and the secondary nucleation events resulting in smaller crystals with increasing misorientations. Consequently, the change of the intra-crystalline organic material level during the crystal switch was also investigated. A proteomic survey allowed us to identify and characterize 149 proteins in Guinea fowl shell. These proteins were quantified at five calcification stages corresponding to the first events of mineral deposition, the growth of calcite units just prior the shift of crystal orientation, then to the period of the deposition of the newly formed crystalline shape and to later stage when the growth of the newly formed crystals is stabilized after the microstructure shift and the secondary nucleation events. We have observed 61 matrix proteins only present in the shift period and potentially responsible of the change of mineral in the shell. Among them are calcium binding proteins (NPNT-X1, CALB3), Protein S100-A6, ANXA1 and 2, CD11b…), core proteins of proteoglycans (TSKU, GP4C…), and other proteins regulating the activity of proteins driving the mineralization (SSP1, GC-1166DF6…). These proteins interact with mineral to produce changes in crystal size and orientation and consequently the new shell structure and its resulting mechanical properties. Data obtained will allow the determination of biological markers that will be used for the genomic selection of chicken layers with improved mechanical shell mechanical properties. Additionally, they provided a list of organic products that will be tested as additives for material and ceramics.

Keywords: Guinea fowl, eggshell, Ultrastructure, Biomineralization, Organic matrix

Identification of Extracellular Vesicles Involved in the Biomineralization of the Hen Eggshell

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The eggshell is a critical barrier against mechanical stresses and microbial penetration. Its integrity is essential to maintain the hygienic quality of this basic human food and to limit the number of downgraded eggs. In such a context, we are looking for eggshell strength specific markers in order to optimize egg quality. The eggshell is made of 95% mineral phase (calcium carbonate on calcite form) and an organic matrix (3.5%) mostly containing proteins. Eggshell formation arises from an extra-cellular biomineralization process, which takes place in a fluid that contains eggshell precursors and involves a transient phase of amorphous calcium carbonate (ACC). This work aims at exploring the presence and the role of extracellular vesicles to stabilize ACC and to address it to the mineralization site. In a first approach, we used real time qRT-PCR to assess the expression of vesicular target genes in several tissues. The results confirmed a high expression of vesicular target genes (edil3, anxa1, anxa2, pcd6ip) in oviduct where mineralization takes place. In this study, we have also explored the role of EDIL3 and MFGE8 proteins in chicken shell at key stages of shell mineralization, and confirmed they could bind Ca2+ and vesicles, thanks to an EGF-like calcium-binding domain and a F5/RC phospholipid-binding domain. It was therefore suggested that both proteins could be involved in the vesicular transport of calcium. In a second approach, electronic microscopy coupled with elementary analysis was used to observe the uterine fluid collected during eggshell biomineralization. Data obtained highlighted the presence of extracellular vesicles (~ 300 nm) containing calcium carbonate. Finally, Western Blot analysis confirmed the presence of EDIL3, a key vesicular protein in the purified vesicle fraction. The results of this study showed for the first time the involvement of extracellular vesicles in the transport of calcified biomaterial for the biomineralization of hen’s eggshell. We proposed a model of calcification using vesicles to stabilize ACC and explaining the fast deposition of the crystalline calcite oriented layer in the shell. The proteins described in this study will have to be explored as biological markers for a selection of chicken layers with improved mechanical properties.

Keywords: Chicken, Eggshell, Biomineralization, Matrix proteins, Vesicles

Feed efficiency and Egg Quality of Mid Laying ISA Brown Hens

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Uniformity in egg production is a critical factor for both hen performance and egg quality. Recent evidence suggests, that significant variation exists in feed efficiency between individual laying hens kept under common housing and dietary conditions. This study aimed to evaluate the internal and external egg quality traits of hens ranked as having high, average or poor feed efficiency. A flock of 450 ISA Brown hens aged 25-weeks, were individually caged and monitored for 6 weeks to calculate feed intake, egg production and feed conversion ratio. Based on average FCR, a sub-population of hens was ranked into high feed efficient, HFE (n = 16; FCR < 1.8), medium feed efficient, MFE (n = 15; FCR = 2.0) and low feed efficient, LFE (n = 16; FCR > 2.1) groups. The hens were monitored for growth performance and FCR from 35 - 40 weeks, eggs were collected from all hens once weekly, and assessed for internal and external egg quality. Hens ranked as HFE, MFE and LFE continued to maintain their FE rankings. Eggs from the hens ranked as HFE had the highest albumen height, Haugh unit, and albumen: yolk ratio (P < 0.001) followed by MFE and LFE hens respectively. On the other hand, hens ranked as LFE produced eggs with a higher yolk percentage (P < 0.001), followed by the MFE and HFE hens respectively. There was no statistical difference observed for egg weight and albumen width of eggs from all FE groups. In conclusion, hens ranked as HFE produced eggs with greater albumen height, Haugh Unit score and albumen: yolk ratio, which are measures of quality, indicating that eggs from HFE hens have a higher quality than eggs from medium and low feed efficient hens.

Keywords: Egg quality, laying hens, performance traits, feed efficiency
Long-term supplementation 25-hydroxyvitamin D3 on bone growth and development, egg production and egg quality in pullets and laying hens

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Skeletal health and egg production are important welfare and economic issues for the layer industry. A study was conducted to evaluate the role of dietary 25-hydroxyvitamin D3 on layer bone 3D structural development and laying performance from 0 to 60 weeks. A total of 390 1-day-old Hy-Line W36 pullets were randomly allocated to 3 treatments with 10 replicates. Dietary treatments were: 1) vitamin D3 at 2,760 IU/kg (D); 2) vitamin D3 at 5,520 IU/kg (DD), and 3) vitamin D3 at 2,760 IU/kg plus 25-hydroxyvitamin D3 (HYD) at 2,760 IU (69μg/kg) (25D). Body weight and feed intake were evaluated at all the end of stage: starter1 (0-3wk), starter2 (4-6wk), grower (7-12wk), developer (13-15wk), prelay (16-17wk), peaking (18-38wk), layer1 (39-48wk), and layer2 (49-60wk).

Egg production was recorded daily, and egg quality was measured every 8wk starting at 18wk. Tibia bone growth rate (BGR) was measured at 10wk using a calcium injection technique. Femurs were scanned using Micro-CT for 3D structural analysis at 17wk and 60wk. The results showed tibia BGR was higher in 25D compared with D at 10wk. At 17wk, 25D treatment showed higher volume in cortical bone, trabecular bone and bone marrow, along with greater cortical porosity, which resulted in a lower cortical bone mineral density (BMD), but without altering bone mineral content (BMC). At wk60, 25D recovered from low cortical BMD, in turn had highest total BMC, and highest cortical volume, and trabecular bone connectivity. For laying performance, DD showed lower feed intake at layer2, but higher at layer3; lower hen day production (HDP) from 18 to 48wk, but a trend of higher HDP during layer5 (P<0.1) compared the others. Meanwhile, 25D had better FCR (F/D)at layer2, and higher HDP during peaking, layer 2, but no changes during later period, which resulted in a higher overall (18-60wk) HDP compared with DD. For the egg quality analysis, at 25 and 33wk, both DD and 25D had higher Haugh Unit compared with D. At 41wk, DD group laid smallest eggs with lower yolk weight, but higher specific gravity and shell thickness compared with the other treatments or D alone, which may partly due to the lower body weight at 40wk. In conclusion, supplementation with 25-hydroxyvitamin D3 stimulated bone growth in pullets, providing more space for mineral deposition during the laying period, as well as improved FCR, cumulative HDP and had positive effects on laying hen bone quality.

Keywords: 25-hydroxyvitamin D3, pullet, laying hen, bone structure, egg production

Egg white powder as gelling and stabilising agent for double emulsions

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Water-in-oil-in-water double emulsions (W/O/W) consist of small droplets of an inner water phase (W) entrapped in oil droplets (O) that are, in their turn, dispersed in another aqueous phase (W). They have the typical structure of oil-in-water (O/W) emulsions but with a reduced fat content, thus resulting interesting for the development of reduced-fat foods. The main issue connected to real applications is the ability to mimic fat behaviour while showing a prolonged stability during storage. Gelling of the internal aqueous phase may be a useful strategy, together with the use of a strong lipophilic emulsifier as polyglycerol polyricinoleate (PGPR). Thus, the aim of this work was to study the effect of egg white powder (EW) as gelling and stabilising agent in double emulsions. In particular, the effects of EW concentration in W1 (0, 5, 10%) as well as volume fraction of W1 (20, 30, 40%) and W2 (40, 50, 60%) on emulsion yield, rheological behaviour, and creaming stability were evaluated. PGPR was used as emulsifier, and a fixed amount of EW (3%) was added in W1. All the samples showed high yields (>95%), meaning that almost all W1 remained entrapped in oil droplets. Creating stability is of paramount importance for double emulsions intended as alternative fats in food formulation, since they have to be prepared beforehand and stored until use. The presence of EW in W1 significantly (p<0.001) increased double emulsion stability after 24 h at 4°C, while the samples with the lowest W1/O (40%) resulted in the lowest stability (68%). Apparent viscosity and pseudoplastic behaviour of double emulsions was significantly affected (p<0.001) by the amount of EW in W1. Increasing EW concentration, more viscous emulsions were obtained, with lower flow index (n) values and higher consistency coefficients (K). In conclusion, the work allowed to improve the knowledge about the effects of inner water phase gelling on double emulsion characteristics, demonstrating that EW can be considered a good stabilizing agent that also improved rheological behavior of these systems.

Keywords: Egg white, double emulsions, reduced-fat food

Rheological behaviours of modified liquid egg albumen with lipase enzyme

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Abstract
Eggs are all-natural and inexpensive sources of high-quality protein and well-balanced basic nutrients. Eggs are processed into liquid for uses in food service industry or ingredients in baking industry due to its excellent functional properties such as whipping, emulsifying and gelling properties. Thus, processed egg products are widely used as a food ingredient in preparation of food service. Egg white is more sensitive to high temperature than whole egg or egg yolk due to the coagulation of protein and adverse affect on functional properties such as foaming properties. Because of the limitation in industry, there is an increasing interest to alternative methods. Nowadays, along with the new practice, processing aids are used. Enzyme-modified liquid egg products are one of the fastest growing products in the egg processing industry due to changing consumer lifestyles that seek out more convenient, quick-to-prepare solutions. Enzyme fermentation has been used in egg products for improving or recovering the technological and functional properties. Lipase enzyme used as processing aids in egg processing to improve functional properties of liquid egg white (LEW). In this research, the rheological behaviours of LEW treated with lipase enzyme at different concentrations (0.1%; 0.2% and 0.3%) were investigated. Rheological characterisation of the enzyme treated and non-treated (control) LEW samples were examined using rheometer. Shear rate ramp test, in the linear viscoelastic region, frequency sweep test, amplitude sweep test and oscillation time tests at 60°C were conducted. In the study, it was determined that with increasing frequency loss modulus (G”) was higher than storage modulus (G’), and therefore the treated LEW samples exhibits liquid-like nature. In enzyme concentration of 0.01% in the low-frequency value (G”> G’) shows viscoelastic property, at the increasing frequency (G’> G”) the protein system exhibits liquid behaviour. In fact, at high frequency with the interclusion of G’ and G” curves it happened that G’=G” and system will act as a viscoelastic solid.

In conclusion, the rheological properties of enzyme modified LEW were determined and this information can be used in egg processing industry. This study showed that lipase enzyme modified LEW exhibited a gel like rheological characteristics with a G” much higher than the G’ and, the Herschel-Bulkley model fit the shear stress and shear rate data.

Keywords: liquid egg albumen, rheological behaviour, lipase, enzyme modified, quality.

Introduction
Eggs provide one of the highest quality proteins and key nutrients. Liquid egg products are widely used in the food industries due to its excellent functional properties such as whipping, emulsifying, and gelling properties (Yüceer and Caner, 2014). Egg albumen in a large number of food processing (such as cakes, muffins, noodles, angel food cakes and bakery products). Liquid egg albumen/white (LEW) should be free from pathogenic bacteria and particularly Salmonella, because of that need to be pasteurized. However, the heat treatments can have detrimental effects on the functional properties of liquid egg proteins resulting in commercially undesirable finished products (Campbell, Raikos, & Euston, 2005). Egg white is more sensitive to high temperature than whole egg or egg yolk due to the possibility of coagulation of protein and adverse effect on functional properties especially foaming properties (Punjadus and McKellar, 1999). Because of the limitation in industry, there is increasing interest to alternative methods, especially in the processing of egg albumen. Nowadays, along with the new practice, processing aids are used. Enzyme modified liquid egg products are one of the fastest growing products in the egg processing industry due to changing consumer lifestyles that seek out more convenient, quick-to-prepare solutions. The use of enzymes during the processing of yolk and whole egg products considerably improves the functional properties in terms of emulsification and solubility. Enzyme fermentation has been used in egg products for improving or recovering the technological and functional properties (foaming, emulsifying and gelling). Lipase enzyme is a part of important processing aid ingredients for egg processing solutions to eliminate egg yolk contamination during processing such as egg breaking & separation process (LEW migrate of fats and phospholipids from yolk to egg white) (Macherey et al., 2011, Macherey, 2007, Kobayashi et al., 1980, Imai, 1976). The emulsifying properties and stability of lipase enzyme treated liquid eggs were substantially improved quality of albumen. Modification of albumen as a result of the treatment with lipase enzyme was reported in previous studies (Macherey, 2007, Macherey et al., 2011, Kobayashi et al., 1980, Imai, 1976). The aim of this study was to investigate the rheological properties of LEW treated with different concentrations (0.01%; 0.02% and 0.03%) of lipase enzyme.
Materials and Methods

Material

Fresh LEW samples were provided from Kskinoglu egg processing plant (Akhisar, Manisa, Turkey).

Enzyme pre-treatment

LEW were treated with the lipase enzyme (Candida cylindracea (ragosa) (Lipomod 34P, Biocatalysts Ltd., Wales, UK) concentration (0.03 w/v %) at 50±0.2 °C for 3 hours. pH of lipase samples was adjusted to 5.00 using citric acid (Tekkim Kimya, Istanbul, Turkey). The LEW treated with different concentrations (0.01%; 0.02% and 0.03%) of lipase enzyme and stored at 4°C.

Rheological analysis

Rheological measurements were carried out in a controlled stress rheometer (DHR-2, TA Instruments, New Castle, DE, USA) using software (Rheology Advantage Data Analysis Program, TA). Parallel plate geometry (40 mm, 1 mm gap) was used for the experiments and plate was equipped with a Peltier temperature control (Ahmed et al., 2003). 1.35 mL of LEW was placed between the parallel-plates using an automatic pipette. All experiments were performed triplicate. Flow ramp test was performed in the presence and absence of lipase enzyme, from low shear rate (0.01 s/s) to high shear rate (100 s/s) during 150 s at 25± 0.01°C. Rheological parameters (shear stress- τ, versus shear rate- γ; apparent viscosity) were obtained from the software. Various rheological flow models based on shear stress-shear rate was tested and the best-fit model was selected based on R² value. Experimental flow ramp curves were fitted to the Herschel-Bulkley model. In order to find the most suitable strain in the linear viscoelastic region, oscillation amplitude test was performed. Test parameters were determined as follows; angular frequency 20 rad/s, temperature 25°C and the %strain varying the value from %0.01 to %100. This test result used in the subsequent analysis and the process is reconstructed for each sample (Sousa and Fernández, 2013). Oscillation frequency test was carried out between the range of frequency 0.01 and 10 Hz at 25°C and using the appropriate % strain value from a previous analysis result (Ahmed et al., 2003, Spencer, 2006). Oscillation temperature ramp was performed from 40°C to 70°C with the heating rate 1°C/minute. %Strain value obtained from previous oscillation amplitude test was used and angular frequency was set 20 rad/s. The coagulation temperatures were determined by extrapolating the rapidly rising storage modulus G’ to intercept the temperature axis (Raikos et al., 2007).

Results

According to the flow ramp results obtained via rheological measurements performed in the study, enzyme treated LEW viscosity values were lower than the control group (Figure 1a). Treated and non-treated LEW samples showed shear thinning behaviour.

Figure 1a. The change of the viscosity with the shear rate. (viscosity curve) Figure 1b. viscoelastic behaviour (elastic modulus-G’, viscous modulus -G”) of examples enzyme modified LEW with frequency.

Flow curves of the enzyme modified LEW fitted by the Herschel-Bulkley model (Figure 1a). By adding lipase enzyme to the LEW, regardless of the amount of concentration, viscosity of the system in control and all samples decreased except lipase with concentration of 0.02% and structure showed shear thinning behaviour. It is noted that decrease the viscosity for thick and thin albumen in literature (Ruth et al., 2013).

Oscillation amplitude curves of control and lipase enzyme modified LEW shown in Figure 1b. The most appropriate stress value in linear viscoelastic region was defined and used in the following analyzes. Viscoelastic characteristics of LEW samples modified with lipase enzyme were determined by applying frequency-scanning test in viscoelastic region and the elastic modulus and viscous modulus values changes with frequency were obtained (Figure 1b). Accordingly, both the elastic modulus and viscous modulus (G”) have been determined that increased depending on the frequency. G’ is reduced due to increase in frequency in the enzyme concentration of 0.01%. In all enzyme concentrations used in the study it is determined that with increasing frequency G” is higher than G’, and therefore obtained solution of the enzyme modified LEW exhibits liquid-like nature. In enzyme concentration of 0.02% in the low frequency value (G”>G’) shows viscoelastic property, at the increasing frequency value (G”<G’) the protein system exhibits liquid behaviour. In fact, at high frequency with the intercalation of G” and G’ curves it happened that G”>G’ and system will act as a viscoelastic solid. According to the obtained data there is no differences between control group and enzyme added LEW in property of gelling and emulsion structure; it can be expressed with the structure of the egg albumen, which has a very good gelling ability.

Frequency sweep curves of the control and lipase enzyme modified LEW sample is given in Figure 2a. G’ (elastic modulus) is a measure of structural integrity, so that when there is a drop in this value, it shows a structural distortion and nonlinear initial. In this regard, high elastic modulus indicate the hardness of the sample, an extensive linear elastic region indicate that the sample maintains its structural integrity against the increased shear stress. In the graph; associated with increased lipase enzyme concentration , loss modulus value decreased and depending on the increased frequencies loss modulus tends to increase. Frequency sweep results showed that the control group had higher G’ and G” values compared to those of lipase enzyme modified LEW. It indicating that the control group has a stronger structure, showing higher resistance to applied frequency-stress.

Figure 2a: Deformation scan curve (oscillation frequency) 2b: Temperature ramp curves of control and lipase enzyme modified LEW sample

Discussion

A Herschel-Bulkley fluid model fitted on rheological behavior
\[\tau = \tau_0 + k \gamma^n\]

where \(\tau\) is shear stress, \(\tau_0\) is the yield stress (Pa); \(\gamma\) is shear rate k; is the consistency coefficient (Pa·s/n), and n is the flow behavior index of Herschel-Bulkley.

For measurement of coagulation temperature, the rheometer was used in oscillation mode. The coagulation temperature was determined by extrapolating the rapidly rising storage modulus G’ to intercept the temperature axis. Control and lipase enzymes modified LEW samples temperature sweep curves was given in Figure 2b. Egg white loses its fluidity around 60 °C. The theromgram of egg white shows 2 major endotherms at 60 to 65 °C, corresponding respectively to the denaturation temperatures of ovotransferrin and ovalbumin (Croguennec et al., 2002). Depending on the increased lipase concentration, loss modulus values increased and depending on the increase in temperature, loss modulus changes was observed.

Conclusion

LEW has the potential to improve rheological behaviour, while obtaining high value-added LEW products and reducing economic loss during storage and uses in food product manufacturing sites. Therefore, with using new methods it is possible improving of quality and functional characteristics of liquid egg. Enzyme modified egg is one of the fastest growing products in the egg processing industry. Also, processing parameters to produce enzyme modified egg products are important. We can conclude that lipase enzyme has stabilised LEW in denaturating temperature range between 60 to 65 °C.

Acknowledgement

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References

Egg is frequently consumed product having various forms such as whole shell, liquid, powder eggs, etc. It is present in many products such as mayonnaise, creams, sauces, and cakes with its emulsifying and foaming properties. It has high quality protein content and also rich in vitamins and minerals. Beside these, egg is also reservoir for Salmonella, particularly S. Enteritidis. EFSA reported more than 100,000 human salmonellosis cases in the European Union (EU) every year. According to the report published by EFSA in 2017, 93.0% of outbreaks associated with eggs and egg products are caused by Salmonella. Salmonellosis is the most important foodborne illnesses and in some cases cause deaths. For this reason, shell egg decontamination is very important to inhibit Salmonella. Various methods have been developed for surface decontamination such as conventional methods (hot-air, hot-water), infrared heating, etc. The heating applications have some limitations since Salmonella is resistant to temperature used in these methods, on the other hand, the egg white is sensitive to heat. While applying the shell decontamination, it is important to maintain the chemical and physical quality of eggs. Application of heat in eggs causes Maillard reactions due to the lysine and glucose contents present in eggs and furosine [6-N-(2-furoylmethyl-L-lysine)], an Amadori compound, is formed with Maillard reaction in early stages. Furosine is accepted as quality parameter mostly in thermally treated products and also considered as egg freshness index. Limited research has been performed about the furosine accumulation in egg products. Therefore, the objective of this study was to compare the furosine formation and changes in the quality properties of fresh eggs and hot water treated eggs. Eggs were treated with hot water for surface decontamination and stored at refrigerator temperature. Furosine content of eggs was determined by the HPLC method after treatment during storage. Also, other physical properties of eggs such as albumen pH, shell color, yolk index, foaming capacity were determined. The results evaluated that the furosine content and other properties of eggs were affected in shell eggs with hot water treatment during storage.

Keywords: decontamination, egg quality, furosine, heat treatment

Assessment of furosine formation and changes in the quality of eggs treated with hot water during storage

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Methionine is classified as the first-limiting amino acid in broilers, since it cannot be synthesized by the chicken itself and because not enough methionine in raw materials of the diet for optimal broiler growth. Therefore, commercial broiler feeds need to be supplemented with one of the three synthetic methionine sources, DL-methionine (DLM), L-methionine (LM) and Hydroxy analog of methionine or HMTBta (2-hydroxy-4-(methylthio) butanoic acid). Hydroxy analog of methionine is a chemically different molecule than D- and L-Methionine, and has a hydroxyl group at the asymmetric carbon whereas DLM has amino group: it is a naturally occurring precursor form of methionine that is absorbed differently within the animal’s body and uses less energy compared to the other methionine sources. Both of HMTBta and DLM are converted to LM and used in the body. However, in practical application of the two methionine sources a controversy regarding the bioequivalence value has been continued for more than 50 years. This controversy and confusion may lead to erroneous feed formulation and increase in feed cost. However, when appropriate methods for comparing bioequivalence of the two products are used more successful inclusion of the two products can be provided. The chemical structure difference leads how and where the two products are absorbed, metabolized and converted to LM in the body. Because of these differences between HMTBta and DLM, when supplemented into diets at grade doses they do not produce dose response curves of the same form. At methionine deficient levels of the response curve, HMTBta fed animals may exhibit lower feed consumption and growth than DLM while at requirement levels they may have greater feed consumption and growth. For example, when nutritionist formulate at normal requirement levels of total sulfur and feeding commercial doses of HMTBta or DLM amino acids (TSAA) they will be able to achieve maximum performance in terms bird growth and feed efficiency. Based on commercial levels and the evidence found in the literature HMTBta has 88% methionine value (on an 100% equimolar basis) while DLM has 99% methionine activity.

Key words: DL-methionine, methionine hydroxy analog, bioequivalence

Implications on bioequivalence of HMTBta and dl-methionine

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Influence of feeding faba beans with high and low vicin-content on the egg quality of two local and one commercial chicken breed

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Faba beans (Vicia faba L.) are cultivated in temperate regions and, due to their high protein content, are deemed an alternative protein source for poultry nutrition to replace soybeans imported from overseas. Local chicken breeds may open a potential to produce eggs and meat with one breed. The main objective of this study was to investigate the effect of feeding of faba beans compared to soybeans on laying performance and egg quality parameters using two local breeds and one commercial purebred layer line. A total of 360 hens of the two local breeds Vorwerkühn (VH) and Brese Gauloise (BG) and the commercial White Rock (WR) line were subjected to three different feeding treatments (in total 40 hens of each breed per feeding group). One group was fed a soybean-based control diet. The other two groups received feed where soybeans were replaced by 20% faba beans containing 1.0% or 0.1% of the anti-nutritive agent vicin respectively. The changeover to the experimental feed took place stepwise in the 18th week of life. 10 eggs per pen were collected in the 34th, 42nd and 50th week of life and analyzed regarding weight of egg, yolk and shell, blood and meat spots, Haugh-units and yolk color. For all egg quality parameters there are significant differences between breeds. WR has the highest egg weight but the lowest yolk percentage and also the brightest yellow color. The local breeds produce smaller eggs with a higher percentage of yolk and less blood spots. The Haugh-units are highest in WR followed by BG and then VH. The diet has only a significant effect on the egg weight in the 34th week of life. During this time, an infestation of the northern fowl mite appeared, the faba bean groups showed significantly lower weights off eggs than the control group. After treatment of the mite infestation no significant differences of the egg weights between all diets were observed. The same applies to the yolk weight. Haugh-units are significantly higher in the group fed vicin-rich faba beans compared to the others. No significant influence of the diet on yolk color and amount of blood spots was detected. The differences between the breeds are caused by the different genetic background and breeding goals of the three breeds. The replacement of soy by faba beans at a level of 20% has no negative effect on the egg quality parameters tested in this study.

Keywords: egg quality, local breeds, faba bean

### Effect of a novel phytase on P content in egg components and on egg production of laying hens

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An experiment was conducted to study the effect of a novel phytase on P content in egg components and on egg production in laying hens. A total of 512 Dekalb White layers were fed 4 experimental maize-SBM-sunflower-based diets starting at 28 weeks of age during 35 days. A Negative Control (NC) diet low in P and without added inorganic P (2,800 kcal AMEn/kg, 162 g CP/kg, 3.4 P/ kg, 2.3 g IP/kg, 40 g Ca/kg) was supplemented with 0, 100 or 200 FTU/kg of phytase/kg diet (Natuphos® E 5000). A Positive Control (PC) diet with 5.4 P/kg diet was prepared with the addition of monocalcium phosphate to NC diet in exchange with only diamin and limestone. Each treatment had 8 replicate cages (2 m2 and wire flooring) with 16 birds/cage. Egg production was recorded during the first weeks. Eight eggs per pen were collected between days 34 and 35 to determine P content in egg components (yolk, albumin and eggshell). Egg yolk and albumen were freeze-dried prior to P determination. All data were analyzed by one-way ANOVA with Tukey’s test for means comparison with a P<0.05 level deemed statistically significant. Compared to NC, the treatment supplemented with 200 FTU/kg NC (PC 200) improved significantly laying rate (89.9 vs. 94.9%), egg weight (61.4 vs. 60.0 g) and egg mass (54.0 vs. 58.3 g/d) and values were similar to the PC. The treatment with 100 FTU/kg had a similar egg weight than PC (61.0 vs. 61.8 g) and a lower egg mass (55.5 vs. 57.9 g/d) due to a lower laying rate. P content in yolk and albumen was similar in all treatments. In all treatments, P content in other diets (0.32-0.35 %) was higher than P content in the egg shell to the level of the PC treatment, while 200 FTU of phytase/kg enhanced egg production performance to the PC level.

Keywords: egg layers, phytase, phosphorus

### The effect of feed structure on egg quality traits of laying hens reared in conventional and enriched colony cages

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The particle size and form of the feed remains often-overlooked aspects of laying hen nutrition. Indeed, the gastrointestinal system can adapt and react morphologically and metabolically to manipulations in diet structure, which may have implications on egg quality in association with improved capacity to digest and absorb available nutrients. Two consecutive experiments were conducted to determine the influence of the hammer mill screen to grind cereals and the form of the diet on egg quality of hybrid white layer hens (Lohmann LSL-Classic). In the former experiment, the influence of feed form (mash, crumbled, and pellet) and screen size (5 mm and 8 mm) on egg quality traits was examined by laying hens using a 3 x 2 factorial arrangement of treatments. The hens (n=864) at 28 weeks of age were randomly divided into 6 dietary treatments, each with 6 replicates of 24 birds over the following 24 weeks period. In the latter study, a total of 640 hens were randomly allotted to 4 dietary treatments arranged in 2x2 factorial with 2 screen sizes (5 mm and 8 mm) and 2 feed forms (mash and crumble). Each diet was fed to 8 enriched cages of 20 hens from 21 to 52 weeks of age. Experimental protocols regarding feed management and housing conditions were the same in both studies except for the cage design. Data obtained from both studies illustrated that egg quality characteristics were barely affected by either screen size or feed form. However, in conventional cages, the consumption of feed in the crumbled and pellet forms decreased the yolk colour score by 4% (P=0.05). Furthermore, when hens kept in colony cages, crumbling layer hen feed, compared with mashing, induced a remarkable (P = 0.001) decrease (12 %) in yolk colour score, a practical measurement that determines intensity of pigmentation of egg yolk. Collectively, these findings indicate the importance of hammer mill screen size on internal and external egg quality traits regardless of the hen rearing system, with the exception of the egg yolk colour score. The significant differences in egg yolk colour score between mash and pelleted or crumbled feeding regimens points to implications of feed processing techniques on carotenoid content in egg yolks or responses by hens to alterations in feed form. The marked decrease in yolk colour score with crumbling warrants further research regarding the carotenoid status of practical diet fed to colony caged hens.

Keywords: Laying hen, rearing system, feed structure, egg quality traits

### Egg quality traits of Isa brown hens fed fonio (Digitaria iburuua)-based diets

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Developing countries including Nigeria are continuously in search of locally available underutilized crops that could increase the feed resource bank. Fonio (Digitaria iburuua) also known as ‘acha’ or ‘hungry man rice’, an indigenous crop of West Africa is gaining attention as an ideal substitute for maize in poultry diets. A 30-week study was conducted to evaluate the effect of fonio grains on the egg characteristics of Isa brown hens. 240, day-old Isa brown chicks were assigned to eight dietary treatments comprising of 0, 15, 30, 45, 60, 75, 90 and 100% fonio grains as replacement for maize. The birds were managed under the same experimental conditions throughout the experiment. Initial body weight and body weight at point of lay were measured. At week 20 of experiment, 12 egg samples were collected daily from each treatment for external and internal evaluations. Data collected were subjected to the one-way ANOVA in a completely randomized design and where significant, means were separated using the Duncan’s multiple range test. Results indicated that there were significant (P<0.05) effects of maize replacement for fonio on some external and internal egg characteristics of Isa brown hens. Significant improvement were observed at 30% fonio compared to maize and other dietary treatments. Shell thickness, hen day production, egg weight, Haugh unit and yolk index were improved by 3.59%, 3.91%, 5.08%, 8.49% and 10.77%, respectively while feed cost had reduced by 14.66% in hens fed fonio diets compared to maize. This study thus demonstrated that fonio can completely replace maize in hen diets and higher egg quality traits are achieved at 30% fonio replacement.

Keywords: Energy source, Egg quality, Performance, Pullets

### The effects of different plant extracts supplemented to layer diets enriched with omega-3 fatty acids on egg efficiency, quality, lipid peroxidation and antioxidant capacity: Antioxidant status and egg sensory evaluation

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Abstract
The aim of this study to investigate the effects of different plant extracts supplemented to layer diets enriched with omega-3 fatty acids on egg sensory characteristics and antioxidant status in blood and liver. A total of 144 28-week old Super Nick genotype
laying hens were randomly distributed into six experimental groups. In trial, six experimental groups were fed diets supplemented without vitamin E or plant extract (Control), supplemented with 200 mg/kg α-tocopherol acetate (Vit-E), supplemented with 5 g/kg grape seed extract (GSE), supplemented with 5 g/kg rosemary extract (RE), supplemented with 5 g/kg olive leaf extract (OLE), respectively. Blood malondialdehyde (MDA) values decreased (P<0.01) with supplementation of GSE and GTE whereas liver MDA values decreased (P>0.01) with supplementation of Vit-E, GSE and OLE to diets. Blood glutathione (GSH) concentration increased with supplementation of GSE whereas liver GSH concentration increased with supplementation of RE, GTE and OLE to diets. Dietary treatments did not influence glutathione peroxidase (GSH-Px) activity of liver and superoxide dismutase (SOD) activity of blood and liver (P>0.01). Sensory evaluation of treatments did not influence glutathione peroxidase (GSH-Px) activity with supplementation of RE, GTE and OLE to diets. Dietary supplemented with 5 g/kg olive leaf extract (OLE), respectively. Blood malondialdehyde (MDA) values decreased (P<0.01) with supplementation of GSE and GTE whereas liver MDA values decreased (P>0.01) with supplementation of Vit-E, GSE and OLE to diets. Blood glutathione (GSH) concentration increased with supplementation of GSE whereas liver GSH concentration increased with supplementation of RE, GTE and OLE to diets. Dietary treatments did not influence glutathione peroxidase (GSH-Px) activity of liver and superoxide dismutase (SOD) activity of blood and liver (P>0.01). Sensory evaluation of eggs prepared in accordance with the trial plan was carried out with ranking test. According to panel scores, the eggs obtained from hens fed with RE diet were the most liked flavor than other dietary treatments. The overall acceptability of eggs from dietary treatments was not different when evaluated by a panel of 12 panelists. In conclusion, plant extracts and vitamin E have potent antioxidant activity without negatively affecting sensory characteristics of the egg.

**Keywords:** antioxidant status, sensory evaluation, laying hens, plant extracts

**Introduction**

Hen eggs enriched with selenium, conjugated linoleic acid, n-3 polyunsaturated fatty acids (PUFA) and vitamin E are considered as functional food or designer eggs on the market in developed countries (Petrovic et al., 2016). However, an increase of the content of bioactive compounds (=phytochemicals).

Such phytochemicals possess to antioxidative benefits in three ways by protecting feed components from oxidative damage, substituting partly the use of α-tocopherol acetate and related compounds and affecting oxidative metabolism in the animal (Wallace et al., 2010). However, different controversial results have been reported on the antioxidative effects of natural plant extracts even in plasma or liver in vivo (rat, Alia et al., 2003; rabbit, Corbi et al., 2018; chicken, Brenes et al., 2008; Vossen et al.; 2011; Karadas et al., 2014) and even in muscle tissues post-mortem (chicken, Laven and King, 2003; Janswil et al., 2015; turkey, Mišnš et al., 2006). Although the inclusion of plant extracts in the feed might be expected to improve the quality of the eggs, there seems to be few reports on the effect of phytochemicals on sensory characteristics of poultry products (Rizza et al., 2008; Botsoglou et al., 2012). Thus, the main purpose of this study is to investigate the effects of different plant extracts supplemented to n-3 enriched egg layer feeds on antioxidant status in blood and liver and sensory characteristics of egg.

**Materials and Methods**

Twenty-eight weeks old one hundred and forty-four (144) Super Nick genotype laying hens were randomly divided into six main groups, and each experiment was replicated four times (6 animal per group). In trial, six experimental groups were fed diets supplemented without vitamin E or plant extract (CONT), supplemented with 200 mg/kg α-tocopherol acetate (Vit-E), supplemented with 5 g/kg grape seed extract (GSE), supplemented with 5 g/kg rosemary extract (RE), supplemented with 5 g/kg green tea extract (GTE), and supplemented with 5 g/kg olive leaf extract (OLE) were formed. All experimental diets contained fish oil (15 g/kg) and flaxseed (39.2 g/kg) to increase n-3 PUFA composition of egg yolk. Diets were formulated to NRC (1994) requirements.

At the end of trial (10th week), 8 hens from each treatment group (2 hen per group) were randomly selected for blood sampling. Three milliliters blood samples were collected from the brachial vein. The activity of SOD in blood and liver were determined according to the procedures described in the commercial kit (Ransod, Randox Laboratories Ltd., UK). The glutathione (GSH) was estimated based on thiobarbituric acid (TBA) reactivity according to the method of Tietze (1969). Lipid peroxidation was estimated based on thiobarbituric acid in plasma or liver contents of bioactive compounds.

Ranking test was used to evaluate the dietary treatments. Sensory evaluation (color, flavor and acceptability) was performed with 12 panelists. They ranked the egg samples in order of preference for the yolk color (1: dark extremely; 6: light extremely), for the flavor (1: strong extremely; 6: mild extremely), and for the overall acceptability (1: bad extremely; 6: like extremely). In conclusion, plant extracts and vitamin E have potent antioxidant activity without negatively affecting sensory characteristics of the egg.

**Table 1: Effects of treatments on MDA, GSH, GSH-Px and SOD activity of blood and liver**

<table>
<thead>
<tr>
<th>Item</th>
<th>CONT</th>
<th>Vit-E</th>
<th>GSE</th>
<th>RE</th>
<th>GTE</th>
<th>OLE</th>
<th>Pooled SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (nmol/mg Hb)</td>
<td>14.99</td>
<td>19.15</td>
<td>14.70</td>
<td>18.94a</td>
<td>1.63</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol/mg protein)</td>
<td>3.53</td>
<td>4.54a</td>
<td>4.08a</td>
<td>3.50</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood (mg/dl)</td>
<td>2.59</td>
<td>3.82</td>
<td>2.80</td>
<td>1.86</td>
<td>1.79</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Liver (mg/dl)</td>
<td>0.24</td>
<td>0.18</td>
<td>0.36</td>
<td>0.42</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood (U/g Hb)</td>
<td>1226.23</td>
<td>1384.18</td>
<td>1037.59</td>
<td>162.67</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (U/mg protein)</td>
<td>319.13</td>
<td>420.38</td>
<td>421.82</td>
<td>306.70</td>
<td>0.178</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood (nmol/g Hb)</td>
<td>1462.71</td>
<td>1540.42</td>
<td>1906.77</td>
<td>2040.95</td>
<td>0.701</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (mg/dl)</td>
<td>51.35</td>
<td>94.40</td>
<td>46.65</td>
<td>57.10</td>
<td>0.219</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Effects of treatments on the results of the Ranking Test**

<table>
<thead>
<tr>
<th>Item</th>
<th>CONT</th>
<th>Vit-E</th>
<th>GSE</th>
<th>RE</th>
<th>GTE</th>
<th>OLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>42</td>
<td>35</td>
<td>37</td>
<td>63</td>
<td>27</td>
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<td>Flavor</td>
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<td>34</td>
<td>42</td>
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<tr>
<td>Acceptability</td>
<td>48</td>
<td>33</td>
<td>41</td>
<td>52</td>
<td>38</td>
<td>39</td>
</tr>
</tbody>
</table>

**Discussion**

Dietary supplementation with n-3 fatty acids is an oxidative stress factor from feeding management. In order to prevent deterioration in animal tissues and consequent oxidative rancidity in final product, natural antioxidants have received a lot of attention. A number of researchers have evaluated the antioxidative activity of alpha-tocopherol acetate (Lin et al., 2005; Basmacoglu-Malayoglu et al., 2009) or plant extracts, including grape seed (Basmacoglu-Malayoglu et al., 2011; Farhat et al., 2017), rosemary (Yesilbag et al., 2011), green tea (Farahat et al., 2016) and olive leaf (Botsoglou et al., 2010; 2012) when supplemented to broiler, layering hens, turkey or quail diets. In response to supplemental plant extract, increased enzyme activities may suggest that birds had greater capacity to clear oxygen free radicals, resulting in lower MDA level. However, it is important to understand that the action of free radicals on antioxidant enzymes could confound the interpretation of results. Free radicals could either depress the concentration or activity of these antioxidative enzymes by activating their damage or increase their concentration by stimulating their induction via endogenous protective mechanism (Salami et al., 2016).

In this study, experimental diets contained 15 g/kg fish oil and 39.2 g/kg flaxseed to increase n-3 PUFA composition of egg yolk. The panelists were not detected off-flavour, which was described as a fish tasty flavor. Researchers (Mohgadasan, 2008; Basmacoglu-Malayoglu et al., 2011; Farhat et al., 2017). The dietary treatments were the following: control (basal diet); Vit-E (basal diet supplemented with 200 mg/kg α-tocopherol acetate); GSE (basal diet supplemented with 5 g/kg grape seed extract); RE (basal diet supplemented with 5 g/kg rosemary extract); GTE (basal diet supplemented with 5 g/kg green tea extract); OLE (basal diet supplemented with 5 g/kg olive leaf extract).

According to the results, two upper entries were 28-56 and lower entries were 32-52 in Kramer and Twiggs’s Table (P<0.05) (Kramer and Twiggs, 1984). The flavor of the sum of the rank was calculated as 31 for eggs obtained from hens fed with GTE diet which was below 32. The color of the sum of the rank was calculated as 63 for eggs obtained from hens fed with RE diet which was above 56 (Table 2).
et al., 2003) suggested that a diet containing less than 50 g of flax seed/kg feed or 15 g fish oil/kg feed will significantly reduce the fishy aromas and flavors in eggs. There is little published information on the results of the plant extracts supplemented to layer diets enriched with n-3 fatty acids on egg sensory characteristics in the literature. In this study, the eggs obtained from hens fed with RE diet were less preferred due to lighter color than other treatments. Similar trend in egg yolk color were reported by Basmacıoğlu et al. (2003) stated that vitamin E (100 ppm) and rosemary extract (500 ppm) did not affect the sensory characteristics (taste and odor) of boiled egg when laying hen fed n-3 enriched diet containing marine algae according to sensory evaluation on a 9-point hedonic scale. Also, according to panel scores, the eggs obtained from hens fed with GTE diet were the most liked flavor than other dietary treatments. Similarly, Ugungayer et al. (2005) assumed that 1.0 and 1.5 % green tea supplementation to the layer diet affected positively in appearance, yolk color and flavor of the boiled eggs according to sensory evaluation on the six point hedonic scale.

In conclusion, plant extracts and vitamin E have potent antioxidant activity without negatively affecting sensory characteristics of the egg. Since the plant extract have dose-dependent antioxidant effects, different doses of each extracts’ should be tried under different stress conditions in further studies.

Acknowledgements

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References


Are omega 3 eggs risky for human health?

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Eggs labeled “omega 3” do contain these fats, which many consumers today associate with a good health. However, depending on which brand buy the omega 3 eggs. Omega 3 eggs in this study, were producing by American farm school, in Thessaloniki and are produced by hens fed a diet contain flaxseed, vitamin E and a mix of aromatic plants (oregano, etc). One omega 3 eggs contain 600 milligrams of ALA (Alpha linolenic Acid) and 160 milligrams of DHA (docosa hexaenoic acid). These eggs start to produced and putting in the market, since 1997. An omega 3 egg per day does not increase the human risk of heart attack or any other type of cardiovascular disease. Four man 53 age years old, which is workers in the farm of these School, consumption boiling 15 eggs per week each for three years and biochemical analysis have demonstrated that not affect, increasing HDL and LDL blood cholesterol. Thus, omega 3 eggs contribute toward better health choice for these and others consumers. Eggs, also, is a balance and nutrient rich inexpensive food, high bioavailable protein, vitamins, choline and lutein which may promote optimal health. However, eggs have not traditionally been regarded as a functional food, primarily due to old concerns by people about adverse effects on serum cholesterol levels. It is now known is not true. It is notice, that the saturated fat in butter, cheese, bacon for example raise your blood cholesterol much more than the cholesterol in your morning egg. So, eat eggs regularly. I didn’t in past, the new knowledge has change the practice.

XVIII European Symposium on the Quality of Eggs and Egg Products

5 Minutes Oral Presentations
The effect of housing system and storage time on cuticle deposition and egg weight of Isa Brown’s eggs

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Czech University of Life Sciences Prague

The aim of this study was to evaluate the differences in cuticle deposition, egg weight and egg weight loss in eggs of laying hybrid ISA Brown housed in three different housing systems (enriched cage, litter and pasture) at fresh eggs and during different storage time (7, 14, and 21 days of storage). The total number of 360 eggs were collected during the experiment. To estimate cuticle, eggs were individually soaked in a MST cuticle blue stain (MST Technologies Ltd., UK) for 1 min and rinsed in tap water 3 times to remove the excess stain then left to dry in room temperature. The eggshell color was measured with L*a*b* color space system (Konica Minolta Inc., Japan). Eggs for egg weight and egg weight loss were individually weighted by a sensitive balance. Regarding to housing system, the results of the study indicated significant effect on all studied parameters. The highest (P <= 0.001) cuticle deposition was observed in eggs from enriched cages (47.51) followed by pasture (44.30) and litter (44.28). Moreover, significantly heavier eggs (P <= 0.001) were produced by hens housed on pasture (60.5 g) compared to litter (58.6 g) and enriched cages (55.7 g). However, the egg grade downgraded with storage time. The fresh eggs showed the highest (P <= 0.001) cuticle values (49.25) while the longest stored eggs (21 days) had the lowest values (42.46). Additionally, from freshness to 21 days old eggs, the egg weight significantly (P <= 0.001) decreased (58.3g / 56.9g respectively). In conclusion, this study further evidence the importance of housing system to be considered for better egg weight and cuticle deposition and therefore safer eggs. Enriched cages were the ideal housing system in terms of cuticle deposition. In addition to the housing system, changes occurring in eggs cuticle layer during storage have a great impact on the egg overall quality.

Keywords: Housing, storage, cuticle, egg weight

Selection for blue eggshell in Country chicken breed
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Blue eggshell color is an autosomal dominant trait in chicken traditionally denoted as oocyan (O), and is a mark for branded eggs. The blue eggshelled country chicken breed in Taiwan usually crossbred with Sige Comb White Leghorn (WL) chicken for commercial use. In order to figure out the change of egg quality after crossbreeding with WL, four strains of blue eggshell layers with different oocyan genotypes and different proportion of white Leghorn bloodline were compared: Ori (0%WL, O/O), Ori-WL (50%WL, O/O), Ori-WL (50%WL, O/O) and NewWL (75%WL, O/O). The egg weight at 30 weeks old among Ori, Ori-WL, New and New-WL are 44.7, 49.7, 49.2 g and 51.2 g and the Haugh unit are 74.5, 78.6, 79.2 and 82.2. The whiteness and chroma of eggshell color at 30 weeks old are 73.4, 80.5, 80.2, 81.8 and 15.1, 12.1, 11.1, 10.1. More blood from WL achieved larger eggs, better egg quality and paler eggshell color.

Keywords: Blue eggshell, egg quality, Country chicken

Physicochemical and Functional Properties of Protease, Lipase and Phospholipase A2 Enzyme-Modified Liquid Egg’s White
Muhammed Yüceer1, Cengiz Caner1
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Processed eggs are used as raw materials in the production of various food products. Pasteurization of liquid egg white (LEW) is commercially performed at thermal treatment. In the U.S., pasteurization requirements for LEW are 55-6°C for a minimum holding time of 30 min. This heat treatment affects the functional and physio-chemical properties of LEW. Yolk contamination and storage condition also very critical in the egg breaking/separation process since it affects foaming properties of liquid egg white (albumen) including foaming capacity and foam stability. In this research, LEW treated separately using protease (0.5%), lipase (0.03%) and phospholipase A2 (0.3%) enzymes with different concentrations were evaluated. The effect of enzymes treatment on physio-chemical and functional properties such as relative whipping capacity, foam stability, color (L*, a*, b*) and pH values were evaluated at 4°C during storage. The properties of enzyme (protease, lipase and phospholipase A2) treatments comparing with non-treated samples were studied. Protease and lipase enzyme treatments increased relative whipping capacities (p<0.05) compared to PL2 treated and non-treated (616.66±28.86) LEA samples, while 0.5% protease enzyme treated liquid egg albumen showed higher RWC values (1000±50) than other groups. However, protease and lipase enzyme treatments RWC values (536.66±41.63 and 780±60.82) decreased during the storage period at day 27, respectively. The use of the protease enzyme was also significantly decreased the lipase, protease and PL2 enzyme results in development of egg functional properties of the modified egg yolk. Enriched housing systems could result in better egg weight and cuticle deposition and therefore safer eggs. Considerably more work will need to be done to determine enzyme effects on the final product.

Keywords: liquid egg white, protease, lipase, phospholipase A2, physico-functional properties

Effect of ultrasound processing on the physico-functional characteristics of liquid egg yolk
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Abstract
Eggs are very nutritious product rich in vitamins, minerals, and high-quality proteins. Egg yolks play important role in production of food products such as mayonnaise, sauces etc. The objective of this research is to investigate the effect of ultrasound application on the physico-functional characteristics of liquid egg yolk. Liquid egg yolk samples were ultrasound treated with 100 and 250 W for 30 seconds of exposure times. Total soluble solids, pH, L*, a*, b* color, concentration was analyzed at weekly intervals during storage at 4°C. The results show that with better physio-chemical properties including stabilizing dry matter value and the color appearance in liquid egg yolk did not change significantly. Furthermore, it was determined that egg pH values decreased with increasing amplitude of ultrasound processing, indicating the efficiency of ultrasound on improving and preserving the functional properties of liquid egg yolk. In this study, the pH value of the egg (6.33 ± 0.03) was decreased with increase in power supplied to samples with the pH of the egg decreased during the storage period. The samples treated with ultrasound remained in the L* value 53 compared to the control group, while the L* values decreased in treatments groups except than 250 W during storage. L* values were between 53,13-54.7. This study shows an overall improvement in physico-functional properties of ultrasound treated liquid egg yolk. Ultrasound treatment has the potential to become a significant breakthrough and improve physico-functional properties of liquid egg yolk during storage.

This study was supported by the Scientific Research Projects Coordinating Department of Canakkale Onsekiz Mart University within the project ID of FBD-2017-1232. Thanks to Dr. Riza Temizkan for the contribution to the study.

Keywords: Egg, liquid egg yolk, ultrasound, quality criteria, storage

Introduction
Eggs are natural and inexpensive sources of unique and well-balanced basic nutrients. Liquid eggs have various functional bioactive compounds which allows it to be considered as unique multifunctional key ingredient in food processing. Thus, it has been used widely in preparation by the foodservice and baking industry due to its excellent whipping, emulsifying, binding and gelling properties (Duan et al., 2017, Techer et al., 2014). Liquid egg should be free from pathogenic bacteria and particularly Salmonella, and because of that need to be pasteurized. However, the heat treatments can have detrimental effects on the functional properties of egg proteins that resulting in commercially undesirable finished products (Campbell, Raikos, & Euston, 2005). Egg is more sensitive to thermal treatment than other foods due adverse effect on functional properties (Punidades and McKellar, 1999). Because of the limitation in industry, there is increasing interest to alternative methods, especially in the processing of liquid egg yolk. Non-thermal methods allow processing of foods below the temperatures used during thermal pasteurization. Ultrasound is one of the techniques that could be the alternative to thermal processing. Ultrasound is well-known novel technique for the improving egg products functional properties and influence the emulsifying properties to obtain value-added egg yogurt for emulsified sauces, mayonnaises, and dressings by increasing firmness of end product (Caner and Yüceer, 2015, Yüceer and Caner, 2018). The uses of ultrasound on egg albumen and whole egg is well-known and studied by many researchers (Yüceer, 2018, Azenemy et al., 2012). However, it remains unknown for the effect of sonication on LEY (liquid egg yolk).

In this study, we have evaluated the physico-functional properties of liquid egg yolk quality. The goal of this Project was to observe the effect of ultrasound treatment on physico-functional properties of LEY. LEY samples were ultrasound treated with 100 and 250 W for 30 sec. of exposure times. The pH value, dry matter and color properties of LEY samples were measured.
Materials and Methods

Material

The fresh liquid egg yolk samples were provided from local market in Canakkale, Turkey. After the eggs were broken, the albumen and yolk were separated from each other’s.

Ultrasound treatment

For ultrasonic treatment, an Ultrasonic Processor UIP1000hd (Hielser Ultrasonics GmbH, Teltow, Germany) with 1000 W of power with BSS215H probe was used. The LEY’s were sunk into an ultrasonic bath and treated with ultrasonic power at 100 and 250 W for 30 sec.

Methods

1. pH measurements

The pH of ultrasound treated and untreated LEY was measured initially and at particular sampling periods in triplicate with calibrated Ohaus Starter 3100 pH meter (Ohaus Corporation, Parsippany, NJ, USA) at 20±2°C and average values were calculated.

2. Color measurements

The color parameters of the ultrasound treated and untreated LEY samples were measured directly with colorimeter (CR-400, Konica Minolta Sensing, Osaka, Japan), calibrated with a white plate as a standard before measurement. The colorimetric analysis results were expressed as color space CIE LAB; L*, a*, b* values as L* (lightness), a* (green to red; higher positive values indicate red color) and b* (blue to yellow; higher positive b* values indicated a more yellowish color) values were reported.

3. Statistical analysis

All data were analyzed using LSM-PROG GLM of the SAS software computer program (SAS Institute, Cary, NC, USA). Differences in samples of experimental data as a result of ultrasound treatments were tested statistically and the values are expressed as mean±standard deviation. All experiments were performed in duplicated with three analyses and the data were calculated employed a two-way analysis of variance (ANOVA, storage time x ultrasound W power) model with Tukey post-hoc comparison test was performed in comparing between treatments and P<0.05 in the study.

Results

Changes in pH of control and ultrasound treated LEY’s samples during storage period at 4°C are presented in Figure 1. The pH values decreased with ultrasound treatment. LEY’s pH increased with increasing ultrasound amplitude %-W. pH values of ultrasound treated LEY’s decreased in storage periods. The pH values of control LEY were 6.33±0.03. The use of the ultrasound was also significantly decreased pH values. Ultrasonication treatments were maintained physic-functional properties of LEY during storage comparing to control group. The reason of pH decreasing may have related to cavitation effect of sonication. This may be also resulted to the fact that egg proteins attain maximum stability at near-neutral pH (Geveke, 2008).

Figure 1. Ultrasound treated/untreated LEY pH values,

Sonicated LEY’s color values during storage period presented in Figure 2a,b,c. The L values of LEY were stable at day 0, but 250W ultrasound treated samples L* value increased significantly at day 17 (Figure 2a). No significant differences in L* values were found ay day 0 in treated samples between treated group samples.

Color properties of ultrasound treated and untreated LEY samples were analysed during storage (Figure 2a and 2b). Ultrasound has decreased a and b values significantly at day 17 (p<0.05). The use of ultrasound has maintained physico-functional properties of LEY during storage. Ultrasonication treatments significantly maintained color stability during storage except 250 W treated samples.

Figure 2: Ultrasound treated/untreated LEY’s a) L* color values, b) a’ color values, c) b’ color values.

Discussion

Physico-functional characteristics of LEY are depends on pH. LEY’s pH is critical for unfolding proteins to isoelectric point and has a potential to improve eggs functional characteristics (Rocculi et al., 2011).

This study shows ultrasound maintained the functional properties of the LEY and it can be used to improvement physico-functional properties. It is possible to restore the stability with treatment of ultrasound.

The presented study highlights that, determining the effect of ultrasound treated LEY, which can be used, in particular in egg processing industry has been reached. LEY has high content of bioactive compound and functionality in food processing. Therefore, liquid egg processors have to maintain, increase or recovery the physico-functional properties of LEY using the ultrasound during processing. The results of study are suitable for industrial-scale application on LEY, while obtaining high value-added LEY products. The ultrasound treated LEY exhibited physico-functional characterization compare with the untreated LEY. Sonication has a potential to become significant breakthrough and improve physico-functional properties of LEY during storage. Understanding the effect of ultrasound of LEY has a great importance for the new product development in egg processing industry. We can conclude from this present study that ultrasound power of 100 W could be used to maintain physico-functional quality without adverse affecting on LEY.

Acknowledgement

This study was supported by the Scientific Research Projects Coordinating Department of Canakkale Onsekiz Mart University within the project ID of FBD-2017-1232. Thanks to Dr. Riza Temizkan for the contribution to the study.

References


The aim of this work was to investigate the effect of Bacillus subtilis PB6 supplementation on the composition of egg yolk lipids, laying hens and broiler breeders under commercial farm conditions. Twenty thousand 57- week-old female broiler breeders and 60,000 broilers were randomly divided into two groups for a seven-weeks farm trial. The main ingredients of basal diets were corn and soybean meal. The first group was fed a basal diet and considered as control group. The second group was fed the basal diet supplemented with the Bacillus subtilis (2 × 107 cfu/kg diet). The birds had free access to water during the whole experiment. The results indicated a highly significant improvement in the fecal score of the group fed with diet contained Bacillus subtilis (P<0.01). Intestinal populations of lactobacilli and E. coli, and fecal moisture were not affected by the supplementation of Bacillus subtilis. Adding Bacillus subtilis PB6 increased the level of total cholesterol in breeders’ serum (P<0.01), however, it decreased the blood heterophil to lymphocyte ratio (P<0.01). It is concluded that Bacillus subtilis PB6 supplementation enhanced the birds’ welfare by improving fecal score and decreasing heterophil to lymphocyte ratio as a stress biomarker.

**Key words:** Bacillus subtilis PB6, broiler breeders, fecal score, welfare

The aim of this study was to determine the effect of using Camelina sativa oil or camelina meal in diets for laying hens on the fatty acid profile of egg yolk lipids. In the experiment, 54 Hy-Line laying hens at the age of 26 weeks were randomly divided into 3 experimental group. The control group (I) received the standard mixture which contained 4% of rapeseed oil. Experimental groups II and III were fed on mixtures containing 4.5% camelina oil and 10% camelina meal, respectively. The hens were maintained in battery cages (two hens per cage), under controlled micro-climate conditions, and were fed ad libitum one of the three isoprotein and isocaloric diet. Birds were fed with experimental diets for a period of 6 weeks. Yolks from three eggs from each treatment were pooled for further analysis. Results of chemical analysis of yolk showed that adding Bacillus subtilis PB6 increased the level of desirable polyunsaturated fatty acid from n-3 family. The content of saturated fatty acid did not change significantly and were at the similar level in all experimental groups. The introduction of 4% camelina oil to the diets for laying hens resulted in increase (P<0.05) in the total polyunsaturated fatty acids belonging to the n-6 and n-3 family decreased significantly (P<0.05) in groups II and III when compared to the control group. Diet supplementation of laying hens with camelina oil as well as camelina meal at a level 4% and 10%, respectively had a favorable effect on the fatty acid profile by increasing the level of desirable polyunsaturated fatty acid from n-3 family.

**Key words:** laying hens, egg yolk, camelina oil, camelina meal, polyunsaturated fatty acids

**Effects of camelina sativa oil or camelina meal in diets for laying hens on egg yolk lipids**

**Sylwia Anna Orzeszewska Dudek, Mariusz Pietras**

National Research Institute of Animal Production, Department of Nutrition Physiology

**Effect of using grape seed oil in laying hen diets enriched in polyunsaturated fatty acids**

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**Abstract**

Grape seed oil is a product with recognized antioxidant capacity due to the rich content of polyphenols. The aim of this study was to evaluate the effects of using grape seed oil in laying hen diets enriched in polyunsaturated fatty acids (PUFA) on the internal and external quality parameters of the eggs. The 4-week feeding trial was conducted on 90 Tetra laying hens (67 weeks). The birds were weighed individually, assigned to 3 groups (C, E1, E2) and housed in digestibility cages. The structure of the basal diet for the 3 groups was with corn (39.65%), wheat (23%), soybean (12.09%) meal, sunflower meal (13.39%) and had 16.72% crude protein and 15.42 MJ/kg gross energy. Compared to the control diet (C), the experimental diets (E1, E2) included 5% fish meal and grape seed oil: 2% (E1) and 4% (E2). The hens had free access to the feed and water. Adding Bacillus subtilis PB6 increased the level of total cholesterol in breeders’ serum (P<0.01), however, it decreased the blood heterophil to lymphocyte ratio as a stress biomarker.

**Key words:** grape seed oil, egg quality, laying hen, PUFA

**Introduction**

Enriched products with polyunsaturated fatty acids (PUFA), like eggs, are currently a trend among consumers, being also one of the most important foods in their daily diet (Wu et al., 2018). One of the major sources of PUFA, available to enrich the omega-3 concentration of eggs and various foods through the bird diets, is flax and its by-products (Vlaicu et al., 2017). However, fats can lead to feed rancidity, which affects their quality (Ren et al., 2013). To prevent this process, it is necessary to use natural antioxidants in diets to improve oxidative stability and their quality. Grape seed oil is a recognized product because of its rich polyphenols content, which gives it the opportunity to improve the quality of the products due to its antioxidant and antibacterial potential (Kiralan et al., 2019). At the same time, grape seed oil also comes with a high intake of unsaturated fatty acids in the products in which it is used (Aypadin et al., 2017).

The purpose of this study was to evaluate the effects of grape seed oil inclusion in laying hen diets enriched in polyunsaturated fatty acids, on the fatty acid profile and on the internal and external quality parameters of eggs.

**Materials and Methods**

The 4-week feeding trial was conducted on 90, Tetra laying hens (67 weeks). Birds were individually weighed, forming 3 experimental groups (C, E1 and E2), homogeneous in terms of body weight. Birds (30 hens/group) were housed in digestibility cages, structured on 3 levels with 10 cages/group. The structure of the basal diet for the three groups included corn, wheat, soybean and sunflower meal. Compared to the control diet (C), the experimental diets (E1, E2) included 5% fish meal and different levels of grape seed oil: 2% (E1) and 4% (E2). The hens had free access to the feed and water. Half lighting was done according to the management guide of the hybrid, with up to 16 hours of light, a microclimate of 21.94 ± 1.96 °C and a humidity of 56.83 ± 6.38%.

Before the production of feeds, samples of fish meal and grape seed meal were collected in order to determine the fatty acid profile. The raw materials studied in this paper was purchased from 2E Prod SRL, Alexandria, Teleorman County. For the grape seed oil, the total polyphenol concentration and antioxidant capacity were determined. Upon feed manufacturing, samples were taken from each group in order to analyse the basic chemical composition. In the end of the trial we collected 18 eggs/group. Measurements
were performed on egg weight, albumen, yolk and eggshell weight (technical balance A&D, GX2000 EC, 0.001 precision); colour intensity (DSM YolkFan); eggshell thickness (Egg Shell Thickness Gauge); eggshell breaking strength (Egg Force Reader). To determine the egg shell life time, Haugh unit values were determined for eggs kept at room temperature for 15 days after the end of the experiment.

The analytical data were compared by variance analysis (ANOV A and t test), using StatView for Windows (SAS, version 6.0). The antioxidative capacity was determined with the DPPH method, proposed by Marxen et al. (2007), using a UV-VIS Analytik Jena spectrophotometer. The results were expressed in mg gallic acid equivalents/g sample (mg GAE/ g sample). The determination of the antioxidant capacity has been done using the DPPH method, proposed by Marxen et al. (2007), using a UV-VIS Thermo Scientific spectrophotometer. The results were expressed in mg gallic acid equivalents/g sample (mg GAE/ g sample).

Results

Table 2 shows the obtained results for the internal and external parameters of the eggs at the end of the experiment.

Table 2. Physical parameters of the eggs

<table>
<thead>
<tr>
<th>Specification</th>
<th>C</th>
<th>E1</th>
<th>E2</th>
<th>P-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weight, (g)</td>
<td>62.48</td>
<td>63.68</td>
<td>63.62*</td>
<td>0.0482</td>
<td>0.249</td>
</tr>
<tr>
<td>- albumen, (g)</td>
<td>38.05*</td>
<td>37.84*</td>
<td>39.47*</td>
<td>0.1885</td>
<td>0.317</td>
</tr>
<tr>
<td>- yolk, (g)</td>
<td>16.33*</td>
<td>16.33*</td>
<td>15.56*</td>
<td>0.1755</td>
<td>0.193</td>
</tr>
<tr>
<td>- eggshell, (g)</td>
<td>8.69*</td>
<td>8.61*</td>
<td>8.47*</td>
<td>0.1989</td>
<td>0.120</td>
</tr>
<tr>
<td>Yolk colour intensity</td>
<td>3.83*</td>
<td>4.56*</td>
<td>3.06*</td>
<td>&lt;0.0001</td>
<td>0.143</td>
</tr>
<tr>
<td>Eggshell thickness, (mm)</td>
<td>0.32*</td>
<td>0.34*</td>
<td>0.33*</td>
<td>0.0385</td>
<td>0.005</td>
</tr>
<tr>
<td>Eggshell breaking strength, (kGF)</td>
<td>3.45*</td>
<td>3.56*</td>
<td>3.67*</td>
<td>0.7262</td>
<td>0.108</td>
</tr>
<tr>
<td>Haugh units (after 15 days at 4°C)</td>
<td>76.65*</td>
<td>77.75*</td>
<td>78.62*</td>
<td>0.8335</td>
<td>1.260</td>
</tr>
</tbody>
</table>

* Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05).

SEM-standard error of the mean.

Table 3 shows the polyunsaturated fatty acid profile according to the level of unsaturation of the collected eggs at the end of the experiment.

Table 3. Fatty acids content of egg samples according to the level of unsaturation (g acid/ 100 g fat)

<table>
<thead>
<tr>
<th>Specification</th>
<th>C</th>
<th>E1</th>
<th>E2</th>
<th>P-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>36.11*</td>
<td>35.24*</td>
<td>33.89*</td>
<td>0.0138</td>
<td>0.335</td>
</tr>
<tr>
<td>MUFA</td>
<td>37.36*</td>
<td>36.71*</td>
<td>33.84*</td>
<td>0.0006</td>
<td>0.470</td>
</tr>
<tr>
<td>PUFAs of which:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- σ-3</td>
<td>26.59*</td>
<td>26.02*</td>
<td>28.25*</td>
<td>&lt;0.0001</td>
<td>0.626</td>
</tr>
<tr>
<td>- σ-6</td>
<td>19.57*</td>
<td>17.34*</td>
<td>20.62*</td>
<td>&lt;0.0001</td>
<td>0.599</td>
</tr>
<tr>
<td>- σ-6/σ-3</td>
<td>15.93*</td>
<td>6.74*</td>
<td>8.96*</td>
<td>&lt;0.0001</td>
<td>0.969</td>
</tr>
</tbody>
</table>

* Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05).

SEM-standard error of the mean.

Discussion

The flax meal, a fatty acid rich raw material, was characterized by a content of 71.59 g PUFAs/100 g fat. The natural antioxidant, grape seed oil, was characterized by 0.25 mg EAG/ g polyphenols concentration, 0.71 mg E/T/ g antioxidant capacity and 56.17 g PUFAs/100 g fat. The regarding data on the main nutrients of feed tested in this experiment (Table 1) reveals that they had a balanced primary chemical composition. Concerning the concentration of fatty acids according to the level of unsaturation of the compound (Table 1), in the experimental groups there was an increase of the PUFAs concentrations by 9.68% (E1) and 18.75% (E2), compared to the control group. At the same time, the σ-3 concentration of the feed was increased due to the inclusion of flax meal. The polyphenols and the antioxidant capacity of the feeds from the experimental groups (E1, E2) were higher compared to the C group. The highest values (P<0.05) of the polyphenol concentration and the antioxidant capacity were recorded in the feed of E2 group (5% flax meal and 4% grape seed oil) compared to the C group. As can be seen in Table 2, the egg weight increased in both experimental groups (E1, E2), compared to the control group, but significantly higher (P<0.05) with 1.92% were the eggs of the E1 group. At the same time, E1 group eggs had the yolk colour intensity significantly increased (P<0.05), compared to the eggs of the control group. Regarding the eggshell thickness and eggshell breaking strength, we can say that both E1 and E2 groups have benefited from an improvement in these parameters significantly (P<0.05).

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In conclusion, the inclusion of flax meal in laying hens diet resulted in a substantial increase of PUFA and PUFA-ω-3 concentrations in feeds and eggs, supported by the antioxidant effect of grape seed oil. The data on egg quality revealed improvements in internal (colour intensity) and external parameters (egg weight, eggshell thickness and eggshell breaking strength).

References


Regarding the egg shelf life, the results presented in Table 2 highlight the fact that the eggs enriched in PUFA n-3, collected from the experimental groups (E1, E2) preserved their organoleptic qualities; thus after 15 days of storage, the Haugh unit recorded higher values than the eggs collected from the control group (C). The obtained results in this study are similar to those in the literature. Criste et al., (2016) evaluated the effect of using grape seed oil (2%) in laying hens diet enriched in PUFA by including 3% flax meal and 3% caromel meal. The use of flax meal resulted in an increase of ω-3 concentration in diets and eggs, compared to C group. At the same time, the recorded results showed that grape seed oil acted as an antioxidant, maintaining the nutritional quality of the eggs in the experimental group. Other researchers (Choupani et al, 2013) who studied the use of 3% grape seed oil in laying hen diets recorded a significant increase in egg weight compared to the eggs of C group. At the same time, the results did not reveal significant differences (P>0.05) in egg production, Haugh unit, egg weight, eggshell thickness and yolk colour intensity.

It is well-known that tannins and polyphenols in general can have beneficial effects on the overall health status of mammals. From the scarce literature available, it seems that small doses of chestnut tannins can be beneficial for poultry gut health and overall performance, but the working mechanism is not well known. This study assessed the effect of hydrolysable chestnut tannins on nutrient oxidation, performance and metabolism in laying hens (Loehmann Brown) of 32 weeks of age. Two basal diets were tested namely a widely used corn-soy based (C) diet and a wheat-rapeseed-palm oil based challenge diet (W) to which chestnut tannins were added (500 mg/kg) (T+) or not (T-) and where two levels of vitamin E were tested (50 mg/kg; E50 and 25 mg/kg; E25), making a total of six different treatments (C/T-E50, C/T+E50, C/T+E25, W/T-E50, W/T+E50, W/T+E25). The vitamin E dose was included as factor to evaluate the antioxidant capacity of the tannins. In total 54 laying hens were housed in digestibility units for 12 days, after which gut content, blood and feces were sampled. No significant increases in digestibility of gross energy and crude protein were seen in groups fed chestnut tannins compared to control groups (C/T-E50 and W/T-E50). Crude fat digestibility was significantly better in groups fed corn-soy based diets with added chestnut tannins (C/T+E50 and C/T+E25) compared to the unsupplemented group (C/T-50) but no effects were seen in laying hens fed the challenge diet (W). A better crude fat digestibility was observed in groups supplemented with tannins and normal vitamin E dose (C/T+E50) compared to tannin supplemented feed with low dose vitamin E (C/T+E25). In wheat-rapeseed-palm oil based diets (W) the metabolisable energy was significantly higher when supplemented with tannins (W/T+E50 and W/T+E25) compared to the control diet (W/T+E50). Other measured parameters such as production traits (body weight, laying rate and feed uptake), feed retention and viscosity of digesta showed no significant differences between groups. It can be concluded that low doses of chestnut tannins improved fat digestibility and metabolisable energy, but that effects depend on the composition of the basal diet.

Keywords: laying hens, chestnut tannins, metabolism, vitamin E

Effect of dietary supplementation of hydroxy-selenomethionine on egg albumen quality

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Egg freshness is one of the most important aspects in the consumer perception, which is often determined by evaluating the albumen quality. The aim of this study was to evaluate the effect of dietary supplementation of hydroxy-selenomethionine (OH-SeMet) in laying hens egg albumen quality under different storage conditions. This trial was performed in a commercial farm using Hy-Line Brown Classic hens of 27 weeks of age at the beginning of the trial. Two free range barns with 25000 hens each were used. The two barns were assigned to one of the two experimental diets: control (C) group, fed a commercial diet with 0.3 mg Se/kg of feed as sodium selenite; OH-SeMet group, fed the same commercial diet plus 0.2 mg Se/kg of feed as OH-SeMet. The feeding trial lasted 38 weeks. Egg albumen quality was evaluated by measuring Haugh units (HU). Every 4 weeks, 200 eggs from each group were collected and analyzed; 20 eggs were immediately analyzed the next day after laying while the rest of the eggs were analyzed after a storage of 5, 9 and 14 days at 10, 20 and 30ºC, respectively (20 eggs for each time and condition of storage). This procedure was replicated 10 times during the 38 weeks of trial. Haugh units decreased significantly (P < 0.01) by increasing temperature and storage time. Significant differences (P < 0.05) were observed between the C group and the OH-SeMet group. Loss of albumen quality was significantly less pronounced (P < 0.05) in the OH-SeMet fed group, in particular when eggs where stored under high stress conditions (14 days at 30ºC). After 9 days of storage at 30ºC, HU decreased from 87.37 to 63.90 in the C group and from 89.33 to 67.07 in the OH-SeMet group, and after 14 days at 30ºC from 87.37 to 57.99 and from 89.33 to 61.64, respectively. The present findings indicate that dietary supplementation of selenium as OH-SeMet showed to improve the maintenance of egg freshness during storage, with the highest impact in warmer seasons.

Keywords: hydroxy-selenomethionine, laying hen, egg freshness
Effect of different storage conditions on the change of egg quality traits of organic eggs

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Abstract

This study was conducted to execute how different storage methods and durations affect quality of organic eggs. A total of 360 eggs collected from Lohmann Brown flock at the age of 80 weeks which the egg quality was lowest were used in the study. After 24 hours of oviposition, 60 eggs were evaluated as fresh egg and quality traits were determined, the other eggs were divided into 2 groups. First group of eggs were stored at room temperature (21-23°C temperature and 55-60% relative humidity) and the second group in refrigerator (4-6°C temperature and 55-60% relative humidity) for 28 days to determine quality traits at different periods. Eggs with covered viols were also divided into 3 groups in both storage conditions. First group had no treatment, second group eggs were washed with warm water (35-40°C) and the third group eggs were covered with plastic stretch wrap without treatment. All eggs were individually weighed before storage. At 7, 14, 21 and 28 days of storage, egg weight, Shell weight, albumen and yolk weight, albumen and yolk color of the egg were determined on 30 eggs. Egg weight loss, albumen index, Haugh unit, yolk index, ratios of albumen, yolk and shell were calculated from these data. The obtained data were compared according to the 2-factor variance analysis method (storage duration: 1, 7, 14, 21, 28 days and storage environment; external environment, external environment with stretched, external environment washed, refrigerator normal, refrigerator stretched, refrigerator washed). Duncan multiple comparison test was used for comparison of means (Ozdamar, 2013). Analyses were performed using SPSS (Version 16) statistical package program.

Results and Discussions

In the study, the effects of storage duration and applications on egg weight, albumen, yolk and shell ratios and weight losses are given in Table 1. Egg weight loss was highest in 28-day storage (4.00%), with 21, 14 and 7-day storage, respectively; 2.89%, 2.07% and 1.12% (P <0.05). The highest storage loss was carried out with 4.24% in the washed eggs kept outside. Even in the case of weight loss assessments, 28 days of storage losses (1.98%) of the eggs in the refrigerator stretched were similar with the loss of the eggs (1.77%) when they were kept in the external environment for only 1 week.

Bell (1996) stated that a weight loss of 1.99% after 1 week of storage at room temperature; Akyurek and Okur (2009) stated that there is a 0.68% loss. Feddern et al. (2017) stated that all the quality traits deteriorated with weight loss in eggs stored for a long time at room temperature. Keener et al. (2006) in the storage of washed and unwashed eggs in the refrigerator conditions similar to the levels obtained in our study determined weight losses.

According to the storage period of the eggs increased in yolk ratio, while the decrease in the ratio of albumen (P <0.05). This is an indication of fluid flowing from the albumen to yolk in the stored eggs. Scott and Silversides (2000) stated that white and brown egg chickens fresh, 1, 3, 5 and 10 days stored at the room temperature in the shell ratio partially decreased, yolk ratio increased and albumen ratio decreased. The increase in shell ratio is due to the total weight loss of the egg originating from albumen during storage period. The groups with the highest losses were the groups where yolk and albumen parts were most frequently changed. Similar findings were found in Sami et al. (2005) due to storage duration and increase in ambient temperature.

The changes in quality traits of albumen and yolk of organic eggs are given in Table 2. Fresh eggs with an initial height of 7.40 mm had a rapid decline when stored for 7 days. This decline was the highest at 28-day storage. In storage applications, the least effect on albumen height was seen in refrigerator applications, and all forms of storage in external environment caused serious decreases (P <0.05). Similar findings were also reported by Jones and Musgrove (2005), and it was stated that there was a constant decrease in the height of the albumen in long term storage at 4 °C. Albumen index values calculated as 8.46 in fresh eggs, decreased down to 28 days storage and decreased to 4.45 (P <0.05). The storage conditions were different from the fresh eggs, but the storage conditions in the refrigerator showed the closest values (P <0.05). The calculated Haugh unit values were determined as 84.69 in fresh eggs and it was seen that the values decreased at 1 week storage rapidly. These decreases reached the highest level in 28-day storage, especially the decrease in the eggs kept in the external environment decreased to B quality (P <0.05). Jones et al. (2018), washed and stored in 40C, unwashed and stored at 22°C at the end of 6 weeks Haugh unit values are determined as 83.1 and 45.5 respectively. It has been reported that the Haugh unit values were significantly reduced in all studies in the storage conditions after 1 week at room temperature (Scott and Silversides, 2000; Sami et al., 2005; Jones and Musgrove, 2005; Keener et al., 2006). Sekeroglu et al. (2016), initially found the Haugh unit to be 94.56 at 10 °C stored eggs. Also, at 7, 14, 21 and 28 days, they stated that 87.64, 84.82, 89.78 and 74.47, respectively. Although albumen pH values were 7.38 in fresh eggs, it increased continuously due to storage period and increased to 9.24 level at 28 days storage (P <0.05).

Introduction

Eggs are one of the most important nutrients in terms of human health with the components it contains. About 12% of the whole egg is protein and an egg contains about 6.5 g of protein (Sarica and Erensoy, 2014). The shelf life of the egg, which is important for human nutrition, depends on the production conditions, the age of the animals and the storage conditions. Eggs are easily degradable (Eke et al., 2013). During storage of eggs, changes in egg yolk and albumen occur due to protein and fat deterioration. These changes in the egg cause to lose some of its nutritional properties (Kralik et al., 2014). Egg quality is influenced by storage temperature, humidity, storage duration and other environmental factors. The most important changes during the storage of eggs occur with the weight loss at albumen pH (Decayere et al., 2001). Eggs may be deteriorated under inappropriate storage conditions (Figuireiro et al., 2013). Therefore, the storage conditions of the eggs are very important in maintaining their freshness. Immediately after laying, CO2, H2 and O2 exchange processes occur depending on the ambient temperature and storage duration of the eggs, and quality decreases rapidly with pH changes (Okeado et al., 2003; Yılmaz and Bozkurt, 2008; Farhad and Fariba, 2011; Chukwuka et al., 2011; Sekeroglu et al., 2016). In preserving the freshness of the eggs, the cuticle layer surrounding the egg shell is the first effective layer. Eggs lose more moisture due to ambient temperature as a result of damage of cuticle layer from collection of eggs to packaging. On the other hand, egg washing, which is carried out in order to eliminate contamination and microbial load in the crust, causes the loss of the cuticle layer (Sarica et al., 2014b). However, due to the storage conditions of the eggs, there is no significant difference in the cuticle layer when appropriate washing is performed in washed and unwashed eggs (Leleu et al., 2011; Gole et al., 2014; Liu et al., 2016).

This study was carried out with the eggs obtained from a flock of brown laying organic production (80-week old). Fresh and 1, 2, 3 and 4 weeks old eggs; room and refrigerator conditions in the first day of the egg was washed or unwashed, and in the case of stretch-covered viols egg weight loss, internal quality traits, the effects of white and yellow pH changes were demonstrated. All comparisons were made to the traits of fresh eggs.

Materials and methods

The study was carried out on 360 daily eggs from a Lohmann Brown laying flock of 80 weeks of age from a firm producing organic eggs. The eggs were kept for 12 hours and their weights were determined individually on a 0.1 g precision scale. After weighing, internal quality traits were measured in 60 fresh eggs and recorded on the first day. The remaining eggs were divided into two groups to determine quality changes in two different environments (external environment and refrigerator). The eggs to be kept outdoors and in refrigerator conditions are divided into three groups. The first group was kept in the cardboard viola without any treatment. The second group was covered with a stretched film so that it did not come into contact with the air with the cardboard viola, and the third group was stored in cardboard viola after being washed. In the washing of the eggs, tap water was used at a temperature of 35-40°C and the washing time was continued for 45 seconds. The temperature of the room where the eggs are kept outside is 21-22°C and the relative humidity is 55-60% by means of data-loggers. The temperature and humidity values in the environment where the eggs were kept in the home refrigerator were recorded with the help of data-loggers and an average of 7.5°C temperature and 54% relative humidity were provided.

At the 7, 14, 21, and 28 days of storage, 15 eggs were collected from the eggs stored in both environment and the weight and internal quality traits of the eggs were determined. At the beginning of the study and during the storage period, egg weight, albumen height, width and length, yolk height, yolk diameter, yolk color, albumen pH and yolk pH were taken (Sarica and Erensoy, 2014; Altan, 2015). The albumen width-length and yolk diameter of the egg were measured by digital caliper.

The obtained data were compared according to the 2-factor variance analysis method (storage duration: 1, 7, 14, 21, 28 days and storage environment; external environment, external environment with stretched, external environment washed, refrigerator normal, refrigerator stretched, refrigerator washed). Duncan multiple comparison test was used for comparison of means (Ozdamar, 2013). Analyses were performed using SPSS (Version 16) statistical package program.
Table 1 Effects of storage duration and conditions of organic eggs on egg weight traits

<table>
<thead>
<tr>
<th>Storage duration (day)</th>
<th>Storage condition</th>
<th>Egg weight (g)</th>
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<th>Albumen ratio (%)</th>
<th>Shell ratio (%)</th>
<th>Weight loss (%)</th>
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Table 2 Effects of storage duration and condition of organic eggs on albumen and yolk traits

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<td>43.98</td>
</tr>
<tr>
<td></td>
<td>Refr. Washed</td>
<td>6.65</td>
<td>6.59</td>
<td>80.05</td>
<td>8.92</td>
<td>17.18</td>
<td>42.98</td>
</tr>
<tr>
<td></td>
<td>External</td>
<td>2.53</td>
<td>1.96</td>
<td>29.82</td>
<td>9.28</td>
<td>12.37</td>
<td>25.77</td>
</tr>
<tr>
<td></td>
<td>External stretched</td>
<td>2.74</td>
<td>2.43</td>
<td>38.60</td>
<td>9.29</td>
<td>12.67</td>
<td>31.03</td>
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<tr>
<td></td>
<td>External washed</td>
<td>3.09</td>
<td>2.61</td>
<td>43.29</td>
<td>9.33</td>
<td>12.00</td>
<td>27.85</td>
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<td></td>
<td>Refr.</td>
<td>5.61</td>
<td>7.02</td>
<td>71.17</td>
<td>9.12</td>
<td>14.61</td>
<td>36.00</td>
</tr>
<tr>
<td></td>
<td>Refr. Stretched</td>
<td>5.58</td>
<td>6.33</td>
<td>71.49</td>
<td>9.09</td>
<td>17.60</td>
<td>42.55</td>
</tr>
<tr>
<td></td>
<td>Refr. Washed</td>
<td>6.43</td>
<td>7.33</td>
<td>78.67</td>
<td>9.30</td>
<td>18.12</td>
<td>44.20</td>
</tr>
<tr>
<td></td>
<td>Means</td>
<td>5.14</td>
<td>6.28</td>
<td>85.90</td>
<td>9.00</td>
<td>15.80</td>
<td>38.36</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.092</td>
<td>0.660</td>
<td>0.011</td>
<td>0.083</td>
<td>0.024</td>
<td>0.072</td>
</tr>
</tbody>
</table>

No significant reductions were observed in yolk height values up to 28 days of storage. Although there was a decline from 17.40 mm to 14.56 mm during 28 days of storage (P<0.05), storage in refrigerator conditions was able to maintain the yolk traits at the level of fresh eggs. Although the yolk index values of fresh eggs, which were calculated as 43.53, decreased due to storage duration (P<0.05). Jones et al. (2018), yolk diameter of the eggs kept at room temperature, yolk index decreased accordingly. Sekeroglu et al. (2016) stated that the yolk index and yolk color of the brown eggs decreased with stored at 7, 14, 21 and 28 days at 10 °C. The yolk color determined as 6.57 in fresh eggs, and a further decrease in the external environment after 7 days of storage (P<0.05), however no significant changes were observed in this feature as well as in other quality traits. Yolk color in organic eggs is lower than the eggs from other production systems, it is stated that the decrease in yellow color is observed in all research results depending on the storage period (Sekeroglu et al., 2016).

Depending on the storage time, the yolk pH values were partial changes according to the fresh eggs (P<0.05) and the yolk pH values ranged from 6.09 to 6.29. External storage conditions were caused by the change in the maximum yolk pH, refrigerator conditions did not change this feature (P<0.05). Similar findings were reported by Scott and Silversides (2000) and Samli et al. (2005) also.
The effect of dietary blackberry and nut leaves on egg quality

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The effect of the dietary blackberry and nut leaves on egg quality was investigated in a 4 week feeding trial on 84, Tetra SL layers (32 weeks). The birds were weighed individually and assigned randomly to 3 groups (C, E1 and E2), housed in cages (2 layers/ cage; 14 cages/group), in an experimental hall with controlled environmental conditions (temperature 23.08±0.98°C; humidity 66.5±5.68%; ventilation/layer 1.70±0.14%) and a reference group. The corn-soybean meal basal diet (2800 kcal/kg ME and 17.8% CP) was the same for all three groups. Unlike the diet formulation for group C, the diet formulations for the experimental groups included: 0.5% blackberry leaves (E1) and 1% nut tree leaves (E2), which increased the level of total polyphenols (mg Gallic acid equivalents/g) to 2.51 (E1) and 2.55 (E2) compared to group C (2.13). Layer performance was monitored throughout the experimental period. The average daily feed intake decreased significantly (P<0.05) in the experimental groups: E1 (103.19 g/ day/layer) and E2 (105.81 g/day/layer) compared to the control group (108.29 g/day/layer). The laying percentage was higher (P<0.05) in groups C and E2 (96.22% for both groups) compared to the experimental group E1 (96.08%). A total of 18 eggs/group were collected in the end of the feeding trial (week 4), and used for the standard yolk samples/group (3 eggs/sample), which were assayed for the concentration of α-linolenic acid, total polyphenols, and for the antioxidant capacity of the yolk. The yolk polyphenol content has been determined by spectrophotometric method; the antioxidant capacity has been done using the DPPH method and the fatty acids were determined by gas-chromatography. Albumin pH decreased significantly (P<0.05) in the experimental groups – 8.18 (E1) and 8.17 (E2) – compared to group C (8.35). Yolk colour intensity (6.14) increased significantly (P<0.05) in group E2 (dietary nut leaves) compared to the control group (5.33). The highest concentration of total polyphenols (0.95 mg/g) and the highest antioxidant capacity (8.61mM acid ascorbic equivalent) were also noticed for E2 yolks. Similarly, the concentration of α-linolenic acid was significantly higher (P<0.05) in the eggs from group E2 (0.647 g /100g total fatty acids), compared to group C (0.597 g /100g total fatty acids). The use of plants with phytoadditive role in layer diets, particularly of the nut tree leaves, has a positive influence on the quality parameters of the eggs.

Keywords: blackberry leaves, nut tree leaves, α-linolenic acid, polyphenols, egg antioxidant capacity
The effect of rosemary volatile oil on performance and egg quality parameters in layer quail diets

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Abstract

The aim of this study was to determine the effects of dietary rosemary and fennel volatile oil supplementation on the laying performance and some egg quality parameters of quails. A total of 120 female Pharaoh quails used in the study were equally divided into three groups containing 40 quails (five replicates of 8 quails each). The study included a control group with no diet additives, and the treatment groups were as follows: 300 mg/kg rosemary volatile oil (Group 1) and 300 mg/kg fennel volatile oil (Group 2). The experiment was lasted 60 days. The feed consumption was significantly (p<0.05) decreased in treatment groups. At the end of the experiment, there was no significant difference in egg weight, egg mass, egg shape index and egg breaking strength between the control and treatment groups. The inclusion of 300 mg/kg rosemary and fennel volatile oil caused a significant (p<0.01) increase in the feed production and improved the yolk color, eggshell thickness and Haugh unit. Fennel volatile oil improved egg yolk colour and eggshell thickness compared to the control group (p<0.001). The Haugh unit was increased in experimental groups I and II compared to the control group (p<0.05). It can be concluded that diet inclusion of laying quails with rosemary and fennel volatile oil is beneficial in improving egg quality characteristics in terms of egg yolk colour, egg shell thickness and Haugh unit. Moreover, supplementation of rosemary and fennel volatile oil in the diets of quails may enhance the egg production. In the light of obtained data, it was concluded that rosemary and fennel volatile oil can be used as natural additives in order to improve the egg quality parameters in poultry rations.

Keywords: Quail, Rosemary, Performance, Fennel, Egg quality

Introduction

The use of antibiotics as growth promoters in animal feed was banned by the Europen Union because of cross-resistance to pathogens and possible residues in tissues. Natural feed additives with plant origins are generally believed to be safer, healtier and less hazardous. Rosemary and fennel aromatic plant and plant extract have antioxidative, antimicrobial and growth-stimulating effects. Rosemary (Rosmarinus officinalis) is an aromatic plant with strong antioxidiant activity in the Lamiaceae family. The majority of the volatile oils in the rosemary are composed of terpene hydrocarbons such as monoterpens, sesquiterpenes, diterpenes, alcohols with their oxygen derivatives, esters, aldehydes and ketones (Carvalho et al., 2005). Fennel (Foeniculum vulgare Mill) Apiaceae family antioxidiant, antimicrobial and antifungal activity has been found in the studies. The main component of the fennel volatile oil (60-70%) is trans-anethole (Florou-Paneri et al., 2005). Many studies have been conducted for the use of aromatic plants alone or combination.

Material and Methods

A total of 120 Pharaoh female quails (Coturnix coturnix japonica) aged 6 weeks were used. The research was carried out in the quail unit of Animal Health Protection, Research and Application Center of Veterinary Faculty of Uludag University. Group feeding was used in all experimental groups. Feed and water were provided ad libitum. The quails were randomly allocated one control and two experimental groups. Each group was randomly divided into 5 subgroups comprised of 8 quails each. The quails kept in laying cages (100 cm wide, 45 cm deep, 21 cm high in front and 17 cm high at the rear to provide 112.5 cm2 per quail) for the duration of the experiment. Rosemary (Experimental group I) and fennel (Experimental group II) volatile oil were added to the experimental groups at 300 mg / kg levels, respectively. In the study, the selection of rosemary and fennel volatile oil doses as 300 mg / kg was taken as a reference from previous studies (Florou-Paneri et al., 2005; Yesilbag et al., 2013). A 24-h constant lighting program was also maintained throughout the experimental period. The study continued for 60 days. Quails were weighed individually at the beginning of the experiment. The feeds were weighed every 14 day to determine feed consumption. The value of feed efficiency was calculated as kg feed per kg and kg feed per one dozen egg. Egg were collected daily and egg production was calculated on daily basis.

The eggs collected every 15 days were stored at room temperature for 24 hours and then weighed on the sensitive scale to determine the egg weight. Egg mass was calculated as given follows (Egg mass = egg weight x egg production/100).

Shape index in eggs: Egg width / egg length x 100 is calculated by the equation.

The eggshell breaking strength was determined with the using of the cantilever system (N/cm²). Albumen height was determined by micrometer. Using these values, Haugh unit was calculated according to the formula (Haugh unit = 100 Log (albumen height+7.57) - (1.7 x egg weight)½) (Haugh, 1937). The shell thickness was measured with a micrometre gauge. Egg yolk colours determined by 1961 Roche Improved Yolk Colour Fan.

Statistical analysis was performed using SPSS (1997) software package for Windows (SPSS, 1997).

Result

The rosemary and fennel oils used in this study were rich volatile oils which composed of a-pinene, terpine, borneol, camphor and cineole in rosemary and trans anethole, fenchone and 1,8-cineole in fennel volatile oil. The effect of dietary rosemary and fennel volatile oil on some performance parameters is presented in Table 1. Feed consumption, feed efficiency and egg production were significantly changed by dietary treatment. Feed efficiency and egg weight and egg significant difference in the egg weight by egg mass in comparison to control and experimental groups. The effects of rosemary and fennel volatile oil on egg quality characteristics are shown in Table 2. There was significant differences yolk colour, eggshell thickness and haugh unit. By contrast, shape index and eggshell breaking strength weren’t significantly different.

Discussion

The addition of aromatic volatile oil to the diet was significantly reduced in feed consumption compared to the control group. Moreover, it has led to a significant improvement in feed efficiency. Bugdayci and Ergun (2011) noted that rosemary volatile oil at the concentration of 200 mg/kg supplementation in broiler diets found a decrease in feed consumption. The decrease in feed consumption value in the experiment may be caused by the irritating effect of the essential oils in relation to the dose. When the results of the study were evaluated, supplementation of fennel volatile oil diet to diet caused a significant increase in egg production compared to the control and experimental group I. The supplementation of rosemary and fennel volatile oil to the quail diets were not found a significant effect on egg weight and egg mass values. In scientific studies, the supplementation of aromatic plants and their extracts which are alone or their mixture results in different performance parameters. Moreover, these differences between the results of the research may depend on the type of volatile oil, composition of the active ingredients, level of addition to the diet, storage conditions of the feed and the composition of the diet. Yolk colour, eggshell thickness and haugh unit were increased with rosemary addition of fennel volatile oil (300 mg/kg). Yesilbag et al. (2011) noted that rosemary and oregano volatile oil mixture (40:160 mg/kg) supplementation had improve the yolk color. Cengiz et al. (2015) determined that the juniper volatile oil suoelementation to the laying quail diets improved the egg yolk color and caused an increase in shell thickness and egg breaking strength. This improvement in yolk colour indicated a passage of carotene, the colouring components of rosemary and fennel volatile oil. In general, the supplementation of rosemary and fennel volatile oils to the quails resulted in a decrease in feed consumption from performance parameters and an increase in egg production and improvement in feed efficiency. Egg production was found to increase in quails fed on a diet to which fennel volatile oil was added.

In terms of egg quality parameters, the supplementation of rosemary and fennel volatile oil to the diet caused an increase in yolk colour, egg shell thickness and haugh unit values. In many scientific studies, the addition of vegetable essential oils to the ration caused an increase in egg quality parameters (Cabu et al., 2003; Botsoglou et al., 2005; Bolukbas et al., 2010; Yesilbag et al., 2013). Therefore, studies reporting that egg quality parameters are not effective (Florou-Paneri et al., 2005; Bozkurt et al., 2012). To obtain different results in many scientific studies based on the addition of extract products supplied from different aromatic plants to poultry diets depends on many factors such as aromatic plant source, level of addition the diet, extraction method, active substance composition and ingredients in diet. For this reason, it should be aimed to determine the antioxidant, antimicrobial and performance enhancing effects of poultry feed by determining the active substance and effective dose in the studies to be carried out thereafter.

In the light of obtained data, it was concluded that rosemary and fennel volatile oil can be used as natural additives in order to improve the egg quality parameters in poultry rations.

References


Table 1. Effects of the addition of rosemary and fennel volatile oil on the performance parameters of laying quail rations (mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Experimental group I (Rosemary volatile oil)</th>
<th>Experimental group II (Fennel volatile oil)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td></td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>5</td>
<td>235.18 ± 2.77</td>
<td>233.21 ± 2.55</td>
<td>0.633</td>
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<tr>
<td>Feed consumption, g/d</td>
<td>5</td>
<td>32.84 ± 1.90</td>
<td>26.86 ± 1.07</td>
<td>0.037</td>
</tr>
<tr>
<td>Egg production, %</td>
<td>5</td>
<td>77.11 ± 1.25</td>
<td>79.75 ± 0.75</td>
<td>0.015</td>
</tr>
<tr>
<td>Egg weight, g</td>
<td>5</td>
<td>10.98 ± 0.08</td>
<td>11.08 ± 0.17</td>
<td>0.793</td>
</tr>
<tr>
<td>Egg mass, g/bird/d</td>
<td>5</td>
<td>8.47 ± 0.17</td>
<td>8.84 ± 0.20</td>
<td>0.001</td>
</tr>
<tr>
<td>Feed efficiency, kg feed/12 egg</td>
<td>5</td>
<td>0.51 ± 0.08</td>
<td>0.40 ± 0.014</td>
<td>0.010</td>
</tr>
<tr>
<td>Feed efficiency, kg feed/kg egg</td>
<td>5</td>
<td>3.59 ± 0.18</td>
<td>3.02 ± 0.10</td>
<td>0.039</td>
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</table>

Table 2. Effect of rosemary and fennel volatile oil addition on egg quality parameters in laying quail rations (mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Experimental group I (Rosemary volatile oil)</th>
<th>Experimental group II (Fennel volatile oil)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td></td>
</tr>
<tr>
<td>Shape index</td>
<td>25</td>
<td>77.95 ± 0.34</td>
<td>77.51 ± 0.33</td>
<td>0.107</td>
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<tr>
<td>Yolk colour</td>
<td>25</td>
<td>9.90 ± 0.06</td>
<td>9.90 ± 0.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Eggshell thickness, µm</td>
<td>25</td>
<td>20.17 ± 0.35</td>
<td>21.08 ± 0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Eggshell breaking strength, N/cm²</td>
<td>25</td>
<td>12.65 ± 0.30</td>
<td>13.11 ± 0.26</td>
<td>0.233</td>
</tr>
<tr>
<td>Haugh unit</td>
<td>25</td>
<td>88.57 ± 0.67</td>
<td>88.50 ± 0.56</td>
<td>0.04</td>
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</table>

*a*: Within a row, values not sharing a common superscript letter are significantly different (p<0.05)
Orange peel oil supplementation in laying quail diet: Effects on performance and oxidative stability of eggs yolk

Metin Çabuk1, Serdar Eratak1, Mehmet Bozkurt2, Buket Gelir1

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In recent years, essential oils have been used as feed additives instead of antibiotics for poultry nutrition. Besides, the effects of essential oils on poultry products are the subject of research. The objective of this study was to investigate the effects of dietary Orange peel Oil supplementation on performance and egg yolk antioxidant status in quail (Coturnix coturnix japonica).

A total of 120 twenty-week old quails were allocated randomly to 1 of 3 dietary treatments. Basal diet and basal diet supplemented with 200 or 300 mg of orange peel essential oil/kg of diet. Each treatment comprised 4 replications with 4 cages (10 quail per cage), amounting to 40 quail per treatment group. Diets (in mash form) and water were provided for ad libitum consumption. To investigate the effect of diet with orange peel essential oil on lipid oxidation of shell eggs during refrigerated storage, four eggs from each replicate collected during the last week of the feeding trial, totalling 16 eggs from each dietary treatment were placed in a refrigerated cabinet at 4 °C to be analyzed for yolk malondialdehyde (MDA) content at 0, 15, 30 days of storage. Dietary supplementation of orange peel essential oil significantly increased egg production compared with control (P< 0.05). Moreover Egg production was the highest in 200 mg/kg orange peel essential oil treatment group. There were no significant differences in feed consumption between treatments, whereas feed conversion ratio was significantly improved by 200 mg/kg orange peel essential oil supplementation. There were no differences between the treatment groups with regard to egg weight. Supplementation of orange peel essential oil to diet had positive effect on oxidative stability of eggs during storage. The extent of lipid oxidation in shell eggs differed among the dietary treatments. Lipid oxidation was higher in the control group compared to all other groups.

Keywords: Laying quail, performance, egg quality, oxidative stability

Calcium pidolate improves egg quality when it is fed to commercial layers from 50 weeks of age

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Calcium pidolate is claimed to have a positive impact on bone quality (in rearing) and to improve egg quality (in lay). Supporting data, however, are quite limited and often relate to studies carried out on small numbers of birds. In this study, we compared egg quality and bone strength in eight commercial free range flocks which received either a standard diet supplemented with 300ppm of calcium pidolate (Treatment n=4) or just the standard diet (Controls n=4) from 50 weeks of age. The results show that there were 0.89% fewer eggs graded as seconds (P < 0.001) in the treatment group and 1.4% increase in eggs graded as large (P < 0.001). A small but significant increase in eggshell breaking strength (P = 0.004), shell weight (p = 0.38) and shell colour (P < 0.001) was also observed. No evidence was found that bone quality at 70 weeks of age was better in the treatment group (tibia or humerus breaking strength, keel bone radiographic density and keel bone deformity scores). It can be concluded from the study that supplementing a layer diet with 300ppm calcium pidolate from 50 weeks of age could be a cost-effective way of maintaining egg quality in longer laying cycles.

Keywords: Layers, eggshell, calcium, pidolate, bones
Quality of functional foodstuffs based on the egg yolk

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“All-Russian Scientific Research Institute of Poultry Processing Industry” – Branch of the Federal State Budget Scientific Institution Federal Scientific Center “All-Russian Research and Technological Poultry Institute” of Russian Academy of Sciences (ARSRIPII)

Growing worldwide interest in functional foodstuffs (FFSs) beneficial for the health of the consumers inspires the development of new products and modern production technologies improving bioavailability and conservation of functional food ingredients (FFIs) to cope with the growing demand for high-quality food. The present study was aimed at the development of new functional eggs, which source was powdered laminaria.

The conservation and bioavailability of FFSs were improved due to the binding of calcium and iodine from the additives by egg proteins. The percentage of calcium bound was 54.1±5.1%, the losses of iodine with the use of this technology were 31.8-38.4% while with other technologies of thermal treatment this parameter can reach 70%. Enriched coagulated melange produced with our technology is also rich in omega-3 PUFAs. The organic form of zinc (complex of zinc with free amino acids and peptides from the ferromolybdate of coagulated egg albumen) was another micro-FFI used for the enrichment of the products. The recipes of two new FFSs were developed: functional coagulated melange with spicy herbs and with olives; these products are enriched with various functional ingredients: -Omega 3; –Lutein; –Immune support, -anti-microbial properties; the functional and substantial (improved texture, binding abilities) properties. Egg products are quick, hygienic. The term “egg products” refers to fresh eggs processed in liquid, dried or frozen eggs, according to stringent hygiene and safety standards obtained by breaking and processing shell eggs. However, more recently there has been substantial growth in further processed egg products. Egg product industry include whole eggs, egg whites, and egg yolks in frozen, refrigerated liquid, and dried forms developed concomitantly with the food industry to provide food manufacturers with safe and ready-to-use eggs. Liquid egg and products are now being widely used by the food service industry. Egg and egg derivatives products are becoming increasingly popular in foodservice due to convenient to use and also provide a cost savings with regards to labor, and use. Use and popularity of innovative processed eggs product is growing in the food industry due to provide functionality at the appropriate price point depending on production capabilities. Both processed eggs and products are growing in usage by the food service industry. Egg product manufacturers have also developed specialty egg products which are formulated and/or cooked eggs intended to mass catering or directly to consumers (Pelletier et al., 2018, Lampkin, 1997).

In an effort to meet consumer demand, egg producers have begun marketing vegetarian, organic, cage-free, free-range, fertile, in-shell pasteurized, and nutrient-enhanced specialty eggs. These eggs offer alternative choices to consumers with special needs and preferences. Due to higher production costs, specialty eggs are usually more expensive than generic shell eggs (Singh, 2005, Anonymous, 2019c).

Innovative eggs and egg products

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Abstract

Eggs are one of nature’s most nutritious foods with functional properties. Egg industry has evolved substantially over time and significant change looking forward. Egg product industry developed new products ready-to-use eggs and provide the market with new egg products which make a difference to the food service market as well as the food industry. These products are becoming increasingly popular in foodservice due to provide a cost savings with regards to labor, storage, and portion control. The popularity of innovative eggs product is growing in the food service industry due to provide functionality at the appropriate price point.

In an effort to meet consumer demand, Egg product industry have begun marketing in frozen, refrigerated liquid (scrambled eggs, egg patties, enzyme modified eggs, omelettes), and dried forms, hard boiled eggs, specialty egg (Nutrient-enhanced specialty eggs, Low cholesterol eggs, Low Yolk (Fat) content, Diabetes eggs, Vegan Eggs, Long Eggs). These eggs offer alternative choices to consumers with special needs and preferences and focus on by developing new types of products which are fast, easy and safe to prepare, without any compromise with regard to health and nutrition.

This review will consider some of the innovative eggs products in the food industry. It will also consider some of the recent innovative reduced cholesterol, high and high options in the egg industry. The use of eggs in functional foods is expected to grow for new food developments. Moreover, Specialty eggs and egg products address new consumer needs and will continue to grow.

Keywords: Egg functional products, designer egg, innovation, new developments, industrial egg products.

Introduction

Eggs are one of nature’s most nutritious foods with functional properties (Yuceer and Caner, 2014). This miracle food offers an assortment of benefits that range from the basic (excellent nutrition, essential calories) to the health-oriented (immune support, anti-microbial properties) to the functional and substantial (improved texture, binding abilities) properties. Egg products are quick, hygienic. The term “egg products” refers to fresh eggs processed in liquid, dried or frozen eggs, according to stringent hygiene and safety standards obtained by breaking and processing shell eggs. However, more recently there has been substantial growth in further processed egg products. Egg product industry include whole eggs, egg whites, and egg yolks in frozen, refrigerated liquid, and dried forms developed concomitantly with the food industry to provide food manufacturers with safe and ready-to-use eggs. Liquid egg and products are now being widely used by the food service industry. Egg and egg derivatives products are becoming increasingly popular in foodservice due to convenient to use and also provide a cost savings with regards to labor, and use. Use and popularity of innovative processed eggs product is growing in the food industry due to provide functionality at the appropriate price point depending on production capabilities. Both processed eggs and products are growing in usage by the food service industry. Egg product manufacturers have also developed specialty egg products which are formulated and/or cooked eggs intended to mass catering or directly to consumers (Pelletier et al., 2018, Lampkin, 1997).

In an effort to meet consumer demand, egg producers have begun marketing vegetarian, organic, cage-free, free-range, fertile, in-shell pasteurized, and nutrient-enhanced specialty eggs. These eggs offer alternative choices to consumers with special needs and preferences. Due to higher production costs, specialty eggs are usually more expensive than generic shell eggs (Singh, 2005, Anonymous, 2019c).

Refrigerated liquid products. Machines break eggs and, if necessary, separate the whites and yolks. Regardless if liquid egg whites are sold refrigerated or frozen, they are always pasteurized for safety and put into package. Egg white based product with vegetables or addressed key consuming needs: Liquid eggs can be enriched with various functional ingredients: - Omega 3; - Lutein; - Vitamins;

Frozen eggs products. These products include separated whites and yolks, whole eggs, blends of whole eggs and yolks or whole eggs and milk and these same blends with salt, sugar or corn syrup added. Frozen egg products have an increased shelf life compared to liquid egg products. Frozen egg products can be used in any formula where shell eggs are required. Frozen products have a wide range from whole eggs to scrambled egg mixes – with whole egg options. These types of egg products are easily integrated into manufacturing systems, including pumping and extrusion (Vladiivl and Christian, 2003, Anonymous, 2019b).

Dried or dehydrated egg products. Known also as egg solids, dried egg products have been produced. Fresh eggs are however, difficult to transport because of their bulkiness, fragility, and highly perishable. Egg in powder form, provides a near complete solution to these problems. Several processing and preservation methods like spray drying, tray drying and freeze-drying techniques have been adopted with repercussions on the qualities of the products (Kumaravel et al., 2012, Asghar and Abbas, 2012).

Specialty egg and egg products. Egg specialties processed for the foodservice industry include wet- and dry-pack, pre-peeked, Low cholesterol eggs, dried egg mixes – either whole, sliced, chopped or pickled; long rolls of hard-boiled eggs; and freeze-dried scrambled eggs.

New liquid egg products: cheesy omelette, spinach and feta omelette and a scrambled egg product. Cardio (Low cholesterol eggs for a healthy heart); the diet of the hen is modified with the addition of special natural nutrients and probiotics. this helps in reducing the cholesterol levels when compared to conventional egg. The levels of cholesterol is less as compared to conventional egg. This is achieved by modification in the feed formula with addition of special natural ingredients such as nutrients and probiotics (Elkin, 2007). However, the separation/removing of cholesterol from liquid egg achieved by adsorption with beta-cyclodextrine, enzymatic degradation, solid extraction etc. (Yüceer et al., 2016, Sun et al., 2011).

Vegan Eggs: But there are already egg alternatives/replacers on the market based on soy and pea protein as egg replacers, but the thing that makes this one special lies in its secret ingredient, black salt instead of artificial flavors to achieve the authentic sulfur-like aroma and taste naturally found in eggs. The vegan egg white is accomplished with silken tofu. That taste along with the realistic texture when cooked, provides the familiar experience of real eggs (Soderberg, 2013).

Long Egg: Hard-boiled eggs come in packaging that resembles a long cookie dough tube. Approximately 20 cm long with a regular center of yolk surrounded by egg white

Conclusions

The potential for new egg products is enormous. Egg products represent a new way to consume eggs meeting the requirements of contemporary consumers. Enjoy eggs because they are tasty, safe to eat, easy to prepare and relatively cheap. While breakfast is dominant time for eggs, other opportunities to enjoy more eggs are being offered. Specialty eggs and egg products address new consumer needs and will continue to grow. Price will continue to be the single most prevalent buying decision for eggs.
This study investigated the efficacy of a single strain *Bacillus subtilis*, hereafter SSB, on the composition ofecal microbiota and its link to the concentration of short chain fatty acids (SCFA) and apparent nutrient retention in pullets and layers. A total of 720, 4-wk old Shaver White pullets were allotted to 1 of 4 corn-soybean basal diet containing either no probiotic (control, CON), 1.1E+08 (Low, LSSB), 2.2E+08 (medium, MSSB) or 1.1E+09 (High, HSSB) CFU/kg of diet. At the end of the grower (wk-10), developer (wk-16) and layer (wk-28) phases, excreta samples were collected for analysis of apparent retention (AR) of components, then eight birds per treatment were euthanized to obtain ceca digesta for SCFA and microbial analysis. Microbial analysis involved high-throughput sequencing of the V3-V4 hypervariable regions of 16S rRNA gene. Regardless of phase and treatment, Firmicutes (44%) and Bacteroidetes (39%) were the predominant phyla. Bacterial diversity decreased (P<0.05) at the developer phase as SSB dose increased, but a distinct clustering pattern (P=0.05) of bacterial community noted at this phase indicated the impact of SSB on bacterial communities. At the genus level, Bacteroides and Faecalibacterium were differentially enriched in the developer phase for SSB- compared with CON-fed birds. Although no differences in microbial diversity were detected in the grower and layer phases, isobutyric acid was elevated in a dose response in the grower (trend, P=0.089) and layer (P=0.034) phases. Predominantly, *Alistipes, Lactobacillus* and *Bifidobacterium* were positively correlated (P=0.05) with AR of most components for SSB-fed birds in the pellet phase. Different species of *Clostridium* (XVIII, XIVa, IV, and XIVb), major butyrate producers, were identified with stronger effect sizes for SSB- compared to CON-fed birds, in all phases. Hence, the results suggest that supplementing chickens’ diet with *Bacillus subtilis* DSM29784 may selectively enrich beneficial bacterial communities, which in turn are critical in promoting growth and performance of hens.

**Keywords**: probiotic, layer, microbiota, SCFA, performance

The effects of licorice root powder (Glycyrrhiza glabra) on microbiological load of hatching eggs and face of laying Japanese quail

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This study was planned to determine the effects of licorice root powder (Glycyrrhiza glabra) on microbiological load of hatching eggs and faces of laying Japanese quails. 240 quails (10 week old) were randomly assigned to four dietary group with four replicate (15 quail). Control group fed basal diet (0 % licorice root powder); other groups were fed basal diet supplemented with 0.5, 1.0 and 1.5% licorice root powder as B, C and D groups respectively. Total mesophilic microorganism, *E coli*, Enterococcus and Staphylococcus aureus loads calculated from control and treated groups of eggs and feces by FDA and BAM methods. As a result total mesophilic microorganism load of quail hatching eggs was found 5.36 log CFU/ml for untreated group and decreased to 2.78 log CFU/ml at C group as a best result. Enterococcus and Staphylococcus aureus loads decreased from 5.46 to 2.72 and 5.29 to 2.70 log CFU/ml at C group respectively. *E. coli* load decreased from 5.13(log) to 2.24 CFU/ml at D group (P<0.05). Total mesophilic microbiorganism load from feces of laying Japanese quails was decreased from 8.68 log CFU/ml to 5.56 log CFU/ml as a best result at B group (P<0.05). Enterococcus and Staphylococcus aureus load decreased from 8.26 to 2.54 and 8.45 to 5.50 log CFU/ml at C group respectively. *E. coli* load decreased from 8.32(log) to 3.20 log CFU/ml at D group (P<0.05). This research shows that Staphylococcus aureus loads of quail hatching eggs and *E. coli* load of feces decreased by 3 log CFU/ml with 1% and 1.5% licorice root powder supplements respectively.

**Keywords**: Microbiological load, glycyrrhiza glabra, licorice, hatching egg, feces
Epidemiological evaluation of reproductive traits and economic of feed and hatchability losses in broiler breeders

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In the present study, Hubbard Classic broiler strain was selected to assess the epidemiological evaluation of reproductive traits and determine the economics of feed and hatchability losses. For this purpose, a complete production data of 20 broiler breeder flocks was selected out of 49 flocks, from 26 to 60 weeks (wk) of age for the years 2005 to 2011 (seven years). Data were collected from the actual computer records saved at commercial breeder farms. Data were collected on weekly basis by a special Performa. The data were evaluated for weekly average egg production percentage, weekly average hatchable egg percentage, weekly average egg weight and weekly average egg hatchability. Number of eggs laid per hen housed, total number of hatchable eggs per hen housed and total number of chicks produced per hen housed during production period (26-60 wk) were also calculated. Feed consumed to produce a hatching egg and one day-old chick per hen was also calculated. Average performances observed from selected flocks were compared with the breed Standards. Annual effect (26-60 wk) were also calculated. Feed hatchable egg percentage, weekly average egg weight and weekly average egg hatchability. Number of eggs laid per hen housed, total number of hatchable eggs per hen housed and total number of chicks produced per hen housed during production period (26-60 wk) were also calculated. Feed consumed to produce a hatching egg and one day-old chick per hen was also calculated. Average performances observed from selected flocks were compared with the breed Standards. Annual effect (26-60 wk) were also calculated.

Keywords: Economic, Hatchability, Epidemiology, Broiler and Broiler Breeder

Effects of rearing systems and age on egg yolk composition of Bovars White layer hybrids

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The purpose of the current study was to investigate the effect of conventional and organic rearing systems and hen’s age (30 and 60 weeks of age) on fatty acid composition, malondialdehyde (MDA), cholesterol and fat soluble vitamins (vitamin A, D, E, K) of egg yolk in Bovars White layer hybrids. A total of 360 eggs, the 90 egg yolks from per group were obtained and, every 9 ones were pooled in 50 mL tubes. The yolks were gently mixed by a glass rod in the tubes and stored until analysis. Ten samples from per group were analyzed with gas chromatography (GC) for fatty acids, the other components were analyzed with high-performance liquid chromatography (HPLC). The total polyunsaturated fatty acids (PUFA), omega-3 (n-3) and omega-6 (n-6) were higher in the organic systems and 30 wks of age groups (P<0.05). The total monounsaturated fatty acids (MUFA) were higher in the conventional system (P=0.05) while it was similar between the age groups. The cholesterol and the retinol (vitamin A) were not influenced by rearing system and age. The MDA, ergocalciferol (vitamin D2) and vitamin K2 were higher in the organic eggs, in contrast, the n-tocopherol (vitamin E) was higher in the conventional eggs (P<0.05). In addition, the MDA and n-tocopherol were higher in the 60 wks of age groups, the ergocalciferol was higher in the 30 wks of age groups (P<0.05). The interaction between rearing system and age was significant (P<0.05). The total saturated fatty acids (SFA), PUFA, n-3, n-6 and vitamin D2 (P<0.05). According to the current study, the PUFA, n-3 and n-6 were higher in the organic eggs. However, the organic eggs were more susceptible to oxidative deterioration due to the high PUFA level in those. It was also determined that the composition of the eggs that obtained from younger hens and organic system were better.

Keywords: Rearing system, age, egg, composition

Implementation of “farm to end-point-consumer in 48 hours” by introducing artificial intelligence (AI)-based Layer-Egg Optimization system

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Over 95% of Taiwanese layer farm still use very traditional practice and with insufficient man power. The resulting eggs may not have the best and consistent quality assurance concerning food safety. Government based production and sales classes are implemented to many of these farms to gather and mix eggs for cleaning the egg surface and mix it. These eggs therefore rarely reach end point consumers in 3 days. Also, these standard practice means loss oforiginating layer farm information, and mixed higher quality eggs with the low-quality ones. Our interdisciplinary, cross institutional Taiwan-UK collaborative team aim to provide a total solution to significantly increase the quality of egg production by developing and implementing the AI-based remote monitoring of environmental factors, layer health monitoring, quality examining, and layer chain management to ensure farm to end-point-consumer within 48 hours. Current achievements and progress include: (1) optimizing cage design based on 3D computational fluid-dynamics; (2) optimizing the distribution and numbers of temperature, ammonium and moisture sensors and automatically link to farm control to homogenate the environmental condition across the animal house; (3) developed robots for feces collection and analysis; (4) developed real-time environmental toxins, bacteria including salmonella, by miniature mass spectrometry within 15 mins; (5) successfully detecting protozoa infection (Caeocoteccidiosis) on the very 1st day of infection to fight against the current 7 days window period with no detection method; (6) realtime recognition of layer health condition via remote body temperature sensing; (7) voice print recognition to monitor respiratory health; (8) clonca infection imaging cognition; (9) egg shell quality cognition; (9) supply chain topology establishment for selling route and distribution optimization; (10) established Bidens pilosa chicken diet to significantly increase laying rate egg weight, egg yolk quality (P <0.05), and can increase layer health and replacement and antibiotic usage. Having these major improvements, we have (11) established Layer Egg Optimization (LEO) grading system and built a website for public education. This all-in-one AI egg system can be distributed in Taiwan for exportation, and be quality and safety of this major protein source.

Keywords: Artificial Intelligence, egg quality, supply chain management, Layer health, Environmental detection

The egg based foodstuff for the prevention of the disorders of carbohydrate and lipid metabolism

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The effectiveness of the prevention of the disorders of carbohydrate and/or lipid metabolism can be significantly improved by the widespread implementation of specialized foodstuffs with hypoglycemic and/or hypolipidemic properties into the diets of the susceptible patient categories. The technology of these products includes the preliminary development of functional ingredients with the expected normalizing effect on fat and carbohydrate metabolism in mammals. Antioxidant properties of polyphenols largely determine their potential pharmacological effects including beneficial effects on carbohydrate and lipid metabolism. However, the use of polyphenols for prophylactic purposes is limited by their low bioavailability. The analysis of scientific literature in the field of clinical nutrition indicated that the main direction in the market of specialized foodstuffs for the prevention or dietary correction of alimentary dependent diseases associated with disturbances of carbohydrate and/or fat metabolism is the development of technological approaches improving the efficiency of polyphenolic compounds within these foodstuffs. The concentration of polyphenols by sorption on protein matrices is a technological approach resulting in “polyphenolic matrices” with high biological activity, increased digestibility and stability. The objective of the study was to properly extract the polyphenols from cranberries and obtain a concentrate from aqueous extract by sorption on the coagulated egg albumen. The coagulated egg albumen was produced by the thermal treatment of the native albumen with regulated pH level using a solution of citric acid as a pH regulator. The coagulated product contained 14.0-14.5% of protein. The trials were performed to optimize the parameters of the sorption of polyphenols from the cranberry extract. The parameters included the concentration of the initial extract, the solution/sorbent ratio, pH of the solution, the temperature and the time of sorption. The rate of the sorption of total polyphenols and anthocyanins on the coagulated egg albumen was determined as the difference in their content in the initial extract and in the supernatant after centrifugation using the Folin-Ciocalteu method and HPLC. The developed functional food ingredient (polyphenolic matrix) can be used in functional foodstuffs for patients with the disorders of carbohydrate and/or lipid metabolism. The study was financed by Russian Science Foundation, grant 16-16-04047.

Keywords: Sorption of total polyphenols and anthocyanins from cranberry, coagulated egg albumen, functional food ingredient, polyphenolic matrix, disturbances of carbohydrate and lipid metabolism
Microbial hazards and risks in industrial Eggs and Meat in Georgia

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This abstract aimed to identify the most relevant microbial hazards and risks for human health in the egg and meat production in Georgia in full production chain. The presence of microbials risks and hazards at different stages in this chain was evaluated as well as potential health effects on consumers related to the major hazards. Poultry production is one of the fastest growing livestock industries because of its advantages in terms of land use and improvements in the food conversion rate of genetically superior poultry breeds. Among the major concerns related to this development are health issues threatening not only animal production, but also the people using the products derived from these animals. Food safety can be defined as the system that keeps food and food products free from substances hazardous to human health. Food safety should be a part of governments strategies to ensure secure food for the consumers. In this context, a “hazard” refers to any biological, chemical or physical property that may cause unacceptable risk (FAO, 1998). The emergence and discovery of new food-borne pathogens and other food-related hazards has increased the need for food-safety measures. The intensification of food production has also changed food processing and handling systems and raised new challenges for food safety institutions. Microbiological risks, such as salmonella-related food poisoning, pesticide residues from feed production, and resistance problems following the use of antibiotics in animal production have become the focus of attention. In the industrial world, legislation and regulations have been implemented, involving both the public and the private sectors. Furthermore, prevention and control measures to reduce the presence of contaminants at different stages in the egg production chain were identified, as well as future developments related to the egg production chain. Some poultry products can pose a higher risk of causing foodborne illness and need to be handled with special care to manage food safety risks. During our researches must be underlines, that Listeria monocytogenes has been recognized as a harmful human pathogen for decades and is known to be an important foodborne pathogen. There have been no documented foodborne L. monocytogenes illnesses due to the consumption of eggs or egg products, even though the bacterium has been isolated from faces, body fluid, and oviducts of asymptomatic laying hens.

Effects of Bacillus subtilis PB6 supplementation on productive performance, egg quality, and hatchability in broiler breeder hens under commercial farm condition

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The aim of this work was to investigate the effect of Bacillus subtilis PB6 supplementation on productive performance, egg quality, and hatchability in broiler breeder hens under commercial farm condition. Twenty thousand 57-week-old female broiler breeders were randomly divided into two groups for a seven-weeks farm trial. The main ingredients of basal diets were corn and soybean meal. The first group was fed a basal diet and considered as control group. The second group was fed the basal diet supplemented with the Bacillus subtilis (2 × 107 cfu/kg diet). Basal diet contained 2800 kcal ME/kg and 13 % crude protein. The birds had free access to water during the whole experiment. The results indicated that dietary supplementation of Bacillus subtilis significantly improved egg production, however, feeding probiotic to breeders decreased the mean egg weight (P<0.05). The body weights of the birds were not affected by the dietary treatments (P>0.05). Bacillus subtilis PB6 supplementation improved the egg haugh unit during 61-63 weeks of age (P<0.05). Adding Bacillus subtilis PB6 lowered dirty and cracked eggs percentage (P<0.05). Hatching egg, egg-shell-thickness, and hatchability significantly increased in breeder hens fed diet supplemented with Bacillus subtilis (P<0.05). It is concluded that Bacillus subtilis PB6 supplementation significantly improved the egg production, egg quality, and hatchability that have economic concern in broiler breeders’ industry.

Keywords: Bacillus subtilis PB6, broiler breeders, egg quality, hatchability
Introduction

During the last decades, the demand for poultry meat, especially further-processed products, has dramatically increased. Consequently, the poultry industry has been pushed to increase breast yield and to produce heavier birds for further processing. However, industry also has to deal with new meat quality issues that seriously affect the taste and nutritional qualities but also the processing ability. As in pork, the technological and sensory defects are often related to variations in energy metabolism, which influence the postmortem pH drop. This variability mainly concerns the breast muscle, whose metabolism is fully glycolytic, and affects color, water holding capacity and texture of the cooked meat. Over the past decade, several myodegenerative anomalies, described as myopathies, have appeared first in countries that use high-yielding strains with high slaughter weights. The main are “white striping”, “wooden breast” and “spaghetti meat” that mainly affect the breast muscle of broilers. The consequences of these anomalies on quality are dramatic because they greatly affect the visual aspect (until rejection by the consumer) but also the processing yields. The poultry industry is looking for solutions related to genetics, nutrition or management practices. The research conducted to date has improved our knowledge of the biological processes involved in the occurrence of meat defects, but for now, no solution has been identified to reduce significantly their incidence without affecting growing performance. We can nevertheless make the assumption that part of the solution will go through genetic improvement, but also (and certainly in addition) nutritional or management solutions. In practical terms, the implementation of these solutions will be facilitated by the development of molecular tools that could allow a deeper phenotypeing of meat defects, ideally on alive birds. This review aims to present recent advances on the search for biological markers, useful for a better screening of broilers affected by meat defects.

Keywords: Meat quality, myopathy, biomarkers, prediction, genomic

Abstract

During the last decades, the demand for poultry meat, especially further-processed products, has dramatically increased. Consequently, the poultry industry has been pushed to increase breast yield and to produce heavier birds for further processing. However, industry also has to deal with new meat quality issues that seriously affect the taste and nutritional qualities but also the processing ability. As in pork, the technological and sensory defects are often related to variations in energy metabolism, which influence the postmortem pH drop. This variability mainly concerns the breast muscle, whose metabolism is fully glycolytic, and affects color, water holding capacity and texture of the cooked meat. Over the past decade, several myodegenerative anomalies, described as myopathies, have appeared first in countries that use high-yielding strains with high slaughter weights. The main are “white striping”, “wooden breast” and “spaghetti meat” that mainly affect the breast muscle of broilers. The consequences of these anomalies on quality are dramatic because they greatly affect the visual aspect (until rejection by the consumer) but also the processing yields. The poultry industry is looking for solutions related to genetics, nutrition or management practices. The research conducted to date has improved our knowledge of the biological processes involved in the occurrence of meat defects, but for now, no solution has been identified to reduce significantly their incidence without affecting growing performance. We can nevertheless make the assumption that part of the solution will go through genetic improvement, but also (and certainly in addition) nutritional or management solutions. In practical terms, the implementation of these solutions will be facilitated by the development of molecular tools that could allow a deeper phenotypeing of meat defects, ideally on alive birds. This review aims to present recent advances on the search for biological markers, useful for a better screening of broilers affected by meat defects.

Keywords: Meat quality, myopathy, biomarkers, prediction, genomic

Molecular tools to predict meat quality

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Abstract

During the last decades, the demand for poultry meat, especially further-processed products, has dramatically increased. Consequently, the poultry industry has been pushed to increase breast yield and to produce heavier birds for further processing. However, industry also has to deal with new meat quality issues that seriously affect the taste and nutritional qualities but also the processing ability. As in pork, the technological and sensory defects are often related to variations in energy metabolism, which influence the postmortem pH drop. This variability mainly concerns the breast muscle, whose metabolism is fully glycolytic, and affects color, water holding capacity and texture of the cooked meat. Over the past decade, several myodegenerative anomalies, described as myopathies, have appeared first in countries that use high-yielding strains with high slaughter weights. The main are “white striping”, “wooden breast” and “spaghetti meat” that mainly affect the breast muscle of broilers. The consequences of these anomalies on quality are dramatic because they greatly affect the visual aspect (until rejection by the consumer) but also the processing yields. The poultry industry is looking for solutions related to genetics, nutrition or management practices. The research conducted to date has improved our knowledge of the biological processes involved in the occurrence of meat defects, but for now, no solution has been identified to reduce significantly their incidence without affecting growing performance. We can nevertheless make the assumption that part of the solution will go through genetic improvement, but also (and certainly in addition) nutritional or management solutions. In practical terms, the implementation of these solutions will be facilitated by the development of molecular tools that could allow a deeper phenotypeing of meat defects, ideally on alive birds. This review aims to present recent advances on the search for biological markers issued from various high throughput methodologies, and whose integration could lead to new diagnostic tools for a better screening of broilers affected by meat defects.

Keywords: Meat quality, myopathy, biomarkers, prediction, genomic

XXIV European Symposium on the Quality of Poultry Meat

Invited Speakers
While the incidence of breast muscle myopathies is strongly increasing, their etiology is still poorly understood and the industry is still waiting for solutions to control them. Literature on the subject has clearly established an unfavorable link with growth and breast meat yield, and the increase in meat pHu also appears to be a predisposing factor. To date, many studies have been conducted to better understand metabolic and structural changes associated with WS and WB defects, but cannot separate the causal mechanisms of the consequences observed at the muscular level. The estimation of genetic parameters for WS and WB defects has demonstrated the existence of a genetic basis for these myopathies (Alnahhas et al., 2016, Bailey et al., 2015). Genetic selection therefore appears to be one of the possible solutions to reduce the incidence of this type of defect, but is insufficiently exploited by the industry, likely because of the lack of measures to estimate sufficiently reliably the degree of damages in affected muscles. In this context, recent works have been initiated, combining genetic, molecular and histological approaches with two major aims, a better understanding of the underlying biological processes and the development of diagnosis tools useful for genetic or genomic selection but also for the evaluation of new rearing and feeding strategies able to reduce the incidence of breast meat defects in broiler.

**Seeking muscle transcripts involved in meat quality variability in broiler**

Several studies have aimed at finding muscle transcripts involved in the control of breast meat quality in broiler. They took advantage of various experimental animal models. They are groups of individuals who are extreme in terms of meat quality from either a unique population or divergent lines selected for meat quality traits. The main traits studied were related to the post-mortem pH drop and more recently to the occurrence of breast muscle myopathies. All these defects influence the physicochemical and functional properties of muscle and a large number of quality parameters such as color, nutritional value, water retention, texture and processing ability (Alnahhas et al., 2014, 2015, Le Bihan-Duval et al., 2008, Petracchi et al., 2019).

Breast meat pHu. As mentioned above there is a clear link between growth and body composition and meat pHu and related meat quality traits (Berri et al., 2001, 2007, Sibut et al., 2008). To overcome these demonstrated links, a divergent selection based on a modern commercial broiler line was made, allowing the creation of two lines diverging specifically for breast pHu (Alnahhas et al., 2014). The analysis of the breast muscle transcriptome of these two lines (named pHu+ and pHu−) revealed very different metabolic statuses and energy production modes between the two lines. The pHu− breast muscles mainly use their high reserve of carbohydrate, while those of the pHu+ line use alternative catabolic pathways leading to significant remodeling of the muscle tissue (Alnahhas et al., 2016, Beauquercq et al., 2016, 2017). From the transcriptome, including 1,436 genes identified as differential between pHu+ and pHu− individuals, APLS models (sparse Partial Least Squares) were adjusted to predict the pHu. The fitted models have good explanatory and predictive ability of the pHu (R²Y = 0.77-0.87, Q² = 0.68-0.79). Twenty-one genes from this model supplemented by 27 other potential candidate genes were selected for high-throughput qRT-PCR validation (Fluidigm technology) on a population of 280 animals from both lines (pHu range 5.41 - 6.50). After a step of elimination of the genes with low explanatory abilities, a final PLS model including 20 confident genes was adjusted, which could be used to predict the pHu of the breast meat with an explanatory (R²) and predictive (Q²) ability of 0.65 and 0.62, respectively and an error rate of 16% (Beauquercq et al., 2017, Figure 1).

![Figure 1: Adjusted PLS model including 20 confident genes used to predict the pHu of the breast meat in broiler (From Beauquercq et al., 2017)](image)

**Breast muscle WS and WB myopathies.** In order to progress in the understanding of the molecular pathways involved in WS and WB and to identify biomarkers that can serve as diagnostic tools for these myopathies, a transcriptomic analysis was performed using an 8×60K Agilent chicken microarray (Pampouille et al., submitted). The analysis included Pectoralis major muscles from three groups: slow-growing animals, fast-growing animals visually free from defects or severely affected by both WS and WB. This study was associated with a fine histological characterization that showed a modification of the muscle architecture induced by selection on growth performance and breast muscle yield, implying fibrosis and adiposis as well as decreased vascularization and greater variation in fiber size suggesting intense regeneration process. Such analysis also allows identifying fine histological phenotypes useful for accurately quantifying the degree of muscle damage. In addition, the transcriptome differential analysis revealed about 10,000 DE genes between the three groups. The Gene Ontology functional analysis has shown that selection for rapid growth and breast meat yield has progressively led to metabolic shift and structural reorganization that constitute favorable environment for the occurrence of myopathies. In particular, this environment may have led to changes in metabolic pathways from a glycolytic metabolism to alternative catabolic pathways to produce energy, which resulted in an adaptive response to oxidative stress as well as early signs of inflammatory processes, cell regeneration and fibrosis. All these processes appear intensifyed in muscles with severe myopathies, in which new mechanisms related to cell defenses and remodeling are also activated. Studying the correlations between gene expression levels and quantitative histological phenotypes using Gene Network Construction (WGCNA) provided original results with the identification of 22 biomarkers whose expression is strongly correlated with the main histological lesions associated with the defects. Indeed, the integrated analysis of molecular and histological data has allowed the identification of a set of biomarkers of myopathies whose interest should now be validated in a larger population and other genetic backgrounds.

**Muscle histological traits: towards new tools for fine typing of breast muscle myopathy**

As mentioned above several immunohistochemical labeling allow to precisely quantifying the lesions associated with the establishment of WS and WB defects in the muscle. This is the case for labeling of connective or adipose tissues, blood vessels and capillaries, or several markers of muscle fiber regeneration (Figure 2). The quantification of these histological phenotypes greatly improves the diagnosis of myopathies as it gives access to much more standardized and precise measures than the visual or palpation-based classification methods currently used by breeders for instance. Currently, the use of histological phenotypes may seem complicated to implement routinely in breeders. Indeed, the histological analysis of the muscles by conventional microscopy and image processing remain long and tedious procedures, difficult to reconcile with the analysis of large numbers. However, recent developments suggest the possibility of performing “medium-throughput” analyzes that can measure very accurately and quickly the amount of fluorescence emitted following the different labeling of muscle lesions involved in the establishment of myopathies in chicken. As for molecular markers, the next step will be to validate on reference populations the interest of using these histological phenotypes to refine the diagnosis of myopathies and evaluate the gain that could be obtained in terms of selection against breast muscle myopathies.

![Figure 2: Examples of immunohistochemical labeling that allow the quantification of lesions associated with the establishment of WS and WB defects in broiler breast muscle (Courtesy of Dr. C. Praud, INRA BOA, Nouzilly, France)](image)

**Finding blood metabolites able to predict meat quality on live animals**

Having confident predictive blood markers would greatly facilitate the development of phenotyping methods in live animals. The divergent lines selected for high or low breast pHu was used to identify blood and muscle metabolite predictors of chicken breast meat pHu. A first step was to analyze by high resolution NMR (proton and phosphorus for muscle and proton for serum) breast muscle and serum extracts from extreme animals belonging to both lines. These analyses revealed very specific metabolomic signatures of the two groups in blood and muscle that enabled an almost perfect discrimination between them (Beauquercq et al., 2016). Twenty and 26 metabolites discriminant between the two lines were identified by multivariate OPLS-DA analysis in serum and muscle, respectively. Three independent models were first fitted with good explanatory (R²Y) and predictive (Q²) abilities for the pHu (R²Y = 0.63 - 0.82, Q² = 0.45 - 0.76). Then, a multiblock model, including muscle and blood metabolites, was subsequently
developed with still better explanatory (R² = 0.91) and predictive (Q² = 0.86) ability. To develop a test that can be routinely used on live animals, the study focused specifically on identified metabolites in the blood. Thus, a model including seven metabolites (acetylglutamine, arginine, formate, glucose, hypoxanthine, phenylalanine and xanthine) still provides good discrimination (R² = 0.73, Q² = 0.64) while limiting as much as possible the number of biological tests for diagnosis (Figure 3). As for muscle transcripts, the predictive potential of this set of serum biomarkers have to be validated on other chicken populations that are representative of the pHi variability observed in slaughterhouses and have different genetic backgrounds from the pHi+ and pHi- lines. If this validation step is conclusive, these biomarkers could be used in selection to exclude from parental stocks the individuals predisposed to produce high-pH or acid meat, or in research to evaluate innovations related to animal husbandry practices.

Figure 3: OPLS-DA model based on seven blood metabolites to predict breast meat pHu (From Beaucerq et al., 2016.)

Towards the identification of mutations involved in the genetic control of breast meat quality

The possibility of having access to the genetic information (pedigree) of animals allows combining positional (QTL) and expressionnal (transcribed) data with two objectives: identifying genetic markers or mutations responsible for the variation of meat quality traits and facilitating the identification of fine molecular phenotypes for diagnosis and selection purposes.

This strategy has already demonstrated its effectiveness in a study on the color of chicken meat. The detection of an expression QTL (or eQTL) confirmed that the gene BCMO1, which encodes β-carotene 15,15'-monooxygenase 1, was responsible for variations in the yellow color of chicken meat and accelerated the identification of causal mutations within its promoter region (Le Bihan-Duval et al., 2011). These results led to the development of a patented genetic test currently available to breeders who wish to control the yellow color of chicken breast meat in response to variations in the composition of feedstuffs. Studies about the interactions with feed have indeed demonstrated the possibility of modulating the deposition of xanthophyll pigments and therefore the coloration of meat through this test (Jalil et al., 2012, 2014).

A similar approach took advantage of the two broiler lines divergently selected for the pHi of the Pectoralis major muscle in order to decipher the genetic control of this trait. By combining detection of selection signatures and QTL and whole transcriptome analysis, it was able to identify genomic regions and a major candidate gene for chicken meat pHu: PPP1R3A. It codes for a muscle-specific regulatory subunit of protein phosphatase 1 (PP1), and by steering the catalytic subunit (pp1A) to glycerin, it promotes dephosphorylation of glycogen synthase (GS) and glycogen phosphorylase (GP) and thereby glycogen synthesis. This gene was differentially expressed between the pHi+ and pHi- lines (Beaucerq et al., 2017) and was located close to the most significant SNP for pHiu (Le Bihan-Duval et al., 2018).

Recently, a GWAS analysis identified QTL regions controlling WS in broiler breast meat (Pamphouille et al., 2018). These results indicated a complex determination of the WS defect with several significant QTL and eQTL acting on the variability of the character in addition to the many other weak-effect genes modeled through the polygenic effect. This study allowed identifying several candidate genes, both positional and functional. The co-location between QTL of WS and eQTL suggests a possible causality of some of these genes. This is the case for LRSAM1, located on chromosome 17, which encodes a ubiquitins protein ligase implicated in various functions, including regulation of signaling pathways and cell adhesion. Mutations in the LRSAM1 gene are responsible in humans for a form of hereditary motor and sensory neuropathy, characterized by progressive distal weakness and muscle atrophy (Guernsey et al., 2010). Interestingly, a cis eQTL for a MYH1F heavy chain implicated in various functions, including regulation of signaling pathways and cell adhesion. Mutations in the LRSAM1 gene are responsible in humans for a form of hereditary motor and sensory neuropathy, characterized by progressive distal weakness and muscle atrophy (Guernsey et al., 2010). Interestingly, a cis eQTL for a MYH1F heavy chain

Figure 4: Identification of the first QTL of white stripping and of a set of 16 positional and candidate genes possibly involved in the control of this defect (From Pamphouille et al., 2018)

Conclusions

Breast muscle myopathies are a major challenge for the broiler industry since they penalize the quality of the meat but also its image. While several solutions have proven effective, such as limiting the rate of growth or the reduction in weight and age at slaughter, they all lead to reducing livestock performance. This leads to exploring the possibilities for genetic improvement of animals, taking advantage of advances made by high-throughput technologies. Although genetic or genomic selection appears to be one of the promising solution to reduce the occurrence of muscle defects, its effectiveness is today penalized by the subjectivity of phenotyping which remains based on a visual notation. The integration of different approaches (histological, transcriptomic and genetic) has made it possible to highlight new indicators, in the form of quantitative histological phenotypes and gene polymorphisms whose expression profiles are strongly correlated with the appearance of the main meat quality defects currently observed in chicken. This opens interesting application perspectives to refine the phenotyping of populations of interest and improve selection efficiency but also to better evaluate the effect of different strategies related to the management or feeding of poultry.

References


**Strategies to reduce incidence of myopathic defects in broiler chickens**

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Abstract

Muscle growth during the juvenile growth phase of chickens (i.e., broilers) is mostly due to hypertrophy of myofibers. Hypertrophy is mostly due to protein accretion, which is a high turnover process that requires a steady supply of metabolic resources, as well as concurrent structural tissue development and support. In spite of complex homeostatic systems, aberrations in repair and/or regeneration can occur in mostly glycolytic pectoral muscles during the rapid growth periods. Breeding efforts to control breast myopathies, described as white striping (WS), woody breast (WB) and spaghetti breast (SB) will be effective, albeit slow, given the low estimated heritability values in broiler chickens. In the meantime, commercial emphasis has been mostly placed on reducing environmental stressors, assuring ample nutrient supply, and modulating growth curve to reduce the severity of lesions at slaughter. Prolonged heat load as a result of increased metabolic heat production and high rearing temperatures may also play a role in the occurrence of myopathic defects. Recent pair-feeding studies indicated that lower rearing temperatures can help reduce the severity of breast myopathies in broiler chickens.

Keywords: Breast, myopathy, temperature

Introduction

Targeted breeding efforts have been extremely successful in the development and widespread utilization of meat-type (i.e., broiler) strains of chickens specifically for poultry meat production. The breeding emphasis on ecologically important white meat (i.e., pectoral muscles) accretion rate and its proportion (yield) to white weight has been particularly effective. The yield of major and minor pectoral muscles now represents nearly 30% of live weight in modern high yield strains. 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, glycolytic (anaerobic) and fast-twitch fibers, with limited vascular supply. Given this unique structure, function and metabolism in a rapidly growing broiler, the homeostatic systems, including the satellite cell-mediated regeneration efforts, may be inadequate at times to sustain and repair the degenerative changes in the breast muscle (Velleman, 2015; Samani, 2009). Sampling (2011) and diffuse ischemic degenerative changes (Sillet, 2015) have been previously reported in both major and minor pectoral muscles of broilers, respectively. Gross and histological changes, as well as meat quality attributes associated with more recent breast myopathies, including white striping (WS), woody breast (WB), and spaghetti breast (SB) have also been extensively described (Petracci and Cavi, 2012; Silvo et al., 2014; Sihvo et al., 2014; Soglia et al., 2016; Lillburn et al., 2018; Aviagen, 2019)

Baiyle et al., (2015) reported that heritability estimates of breast myopathies were low suggesting non-genetic or environmental factors contributing to their etiology (Aviagen, 2019). There has been a high degree of variation in the incidence and severity of breast myopathies among the farms within the same production complex, among the multiple production facilities within a farm, and even within a production facility. Thus, commercially much effort has been placed on reducing the severity of these myopathies. Breeding management to eliminate extremes in air quality (low oxygen levels; elevated carbon monoxide levels during the brooding period), adjustments in the level and bio-availability of amino acids and essential minerals; viruses, viroids and mycoplasmas; vaccines; stressors; and support. In spite of complex homeostatic systems with limited vascular supply, assuring ample nutrient supply, and modulating growth curve to reduce the severity of lesions at slaughter. Prolonged heat load as a result of increased metabolic heat production and high rearing temperatures may also play a role in the occurrence of myopathic defects. Recent pair-feeding studies indicated that lower rearing temperatures can help reduce the severity of breast myopathies in broiler chickens.

Keywords: Breast, myopathy, temperature

**Targeted breeding efforts have been extremely successful in the development and widespread utilization of meat-type (i.e., broiler) strains of chickens specifically for poultry meat production. The breeding emphasis on ecologically important white meat (i.e., pectoral muscles) accretion rate and its proportion (yield) to white weight has been particularly effective.** The yield of major and minor pectoral muscles now represents nearly 30% of live weight in modern high yield strains. 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, glycolytic (anaerobic) and fast-twitch fibers, with limited vascular supply. Given this unique structure, function and metabolism in a rapidly growing broiler, the homeostatic systems, including the satellite cell-mediated regeneration efforts, may be inadequate at times to sustain and repair the degenerative changes in the breast muscle (Velleman, 2015; Samani, 2009). Sampling (2011) and diffuse ischemic degenerative changes (Sillet, 2015) have been previously reported in both major and minor pectoral muscles of broilers, respectively. Gross and histological changes, as well as meat quality attributes associated with more recent breast myopathies, including white striping (WS), woody breast (WB), and spaghetti breast (SB) have also been extensively described (Petracci and Cavi, 2012; Silvo et al., 2014; Sihvo et al., 2014; Soglia et al., 2016; Lillburn et al., 2018; Aviagen, 2019)
Materials and Methods

Study Design and Management: Male broilers of a fast-growing and high yield broiler strain were reared in 12 environmentally controlled rooms (3.1 by 3.7 m) to 54 d of age. Eight pens were maintained under thermoneutral temperatures (Normal Temp; NT) and the remaining 4 pens under warm temperatures throughout rearing (Warm Temp; WT) period. Birds in four N pens were pair-fed the same amount of feed as reared under WT treatment consumed (pair-fed to same at normal temperature; PNT Treatment). Temperatures in the NT treatment were 30-32°C during week 1, 27-29°C during week 2, 25-27°C during week 3, and 20-26°C thereafter. Temperatures in the WT were 29-31°C throughout the trial. Each pen was equipped with an electric heater, exhaust fan, evaporative cooler, and ceiling fan to maintain appropriate temperatures. High and low temperatures for each room were recorded and adjustments to reheatings programs were made. Lighting programs were set to 23L:1D from 1 to 7 d and 43 to 56 d, and 20L:4D from 8 to 42 d, with pens as experimental units for live production and processing performance variables, and birds as experimental units for blood chemistry variables. All percentage data were transformed to arc sine values before analysis, and Tukey’s test was used to compare and separate means when main effects were significant (P < 0.05).

Results and Discussion

Significant (P<0.001) treatment effects were detected for body weight (BW), feed consumption (FC), feed conversion (FCR) and BW uniformity (Table 1). Compared to WT, BW was reduced by about 28% at 49 d and compared to NT at 54 d. Broilers reared under PNT outperformed (higher BE and lower FCR) those reared under WT, although they consumed similar amount of feed. No effect on total mortality was observed due to temperature treatments (P>0.05). These observations are consistent with those paired-feeding studies reported by Ma et al. (2018). BW uniformity was significantly reduced with WT at 54 d mostly due to numerically higher mortality.

As expected, T increased under both WT, at 49 and 54 d, but showed a reduction in broilers reared under PNT (Table 2-3). Blood chemistry responses in control birds were consistent with adequate capillary supply in broiler breast muscle in relation to productivity and ascites. Meat 

References


pH muscle pH was determined 15 min post-slaughter (fillet), minor (tender), and total breast available) and then crated for 6 h to simulate pre-slaughter lairage. Six randomly selected Xpress Analyzer. Blood chemistry parameters of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply and tissue of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply and tissue of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply and tissue of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply and tissue of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply and tissue of pectoral muscles (Hoving-Bolink et al., 2000) and satellite cell signaling (Christov et al., 2007). Poor vascular supply.


MELOCHE, K.J., FANCHER, B.J., EMMERSON, D.A., BILGILI, S.F. and DOZIER, W.A. III (2018b) Effects of reduced dietary energy and amino acid density on Pectoralis major myopathies in broiler chickens at 36 and 49 days of age. Poultry Science http://dx.doi.org/10.3382/ps/pey454


Table 1. Treatment effects on growth performance at 54 d of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Feed Consumption (g)</th>
<th>FCR (g:g)</th>
<th>Total Mortality (%)</th>
<th>BW Uniformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>4642a</td>
<td>6715a</td>
<td>1.83b</td>
<td>2.4</td>
<td>89a</td>
</tr>
<tr>
<td>WT</td>
<td>3354b</td>
<td>5641b</td>
<td>2.08a</td>
<td>7.9</td>
<td>55b</td>
</tr>
<tr>
<td>PNT</td>
<td>3669b</td>
<td>5383b</td>
<td>1.79b</td>
<td>2.4</td>
<td>95b</td>
</tr>
<tr>
<td>SEM</td>
<td>45.6</td>
<td>125.0</td>
<td>.015</td>
<td>1.44</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.001).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.

Table 2. Body temperature and blood chemistry at 49 d of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temp (°C)</th>
<th>pCO₂ (mm Hg)</th>
<th>TCO₂ (mmol/L)</th>
<th>pO₂ (mm Hg)</th>
<th>PCV (%)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>Glucose (mg/dL)</th>
<th>pCK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>41.8b</td>
<td>45.8a</td>
<td>29.0a</td>
<td>36.4a</td>
<td>22.9a</td>
<td>27.6a</td>
<td>233b</td>
<td>66,406a</td>
</tr>
<tr>
<td>WT</td>
<td>42.9a</td>
<td>39.0b</td>
<td>25.4c</td>
<td>37.6a</td>
<td>21.1a</td>
<td>24.3a</td>
<td>254a</td>
<td>30,602b</td>
</tr>
<tr>
<td>PNT</td>
<td>41.2c</td>
<td>44.5a</td>
<td>27.8b</td>
<td>44.0a</td>
<td>23.3a</td>
<td>29.7b</td>
<td>231b</td>
<td>14,569c</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>1.27</td>
<td>0.40</td>
<td>0.41</td>
<td>0.23</td>
<td>0.37</td>
<td>2.1</td>
<td>2.968</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.001).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.
3Packed cell volume.

Table 3. Body temperature and blood chemistry 55 d of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temp (°C)</th>
<th>pCO₂ (mm Hg)</th>
<th>TCO₂ (mmol/L)</th>
<th>pO₂ (mm Hg)</th>
<th>PCV (%)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>Glucose (mg/dL)</th>
<th>pCK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>41.6b</td>
<td>7.36b</td>
<td>50.3b</td>
<td>48.1b</td>
<td>28.4b</td>
<td>23.2b</td>
<td>26.6b</td>
<td>101,263a</td>
</tr>
<tr>
<td>WT</td>
<td>42.5a</td>
<td>7.44a</td>
<td>34.7a</td>
<td>24.5</td>
<td>21.5a</td>
<td>25.3a</td>
<td>30,602b</td>
<td></td>
</tr>
<tr>
<td>PNT</td>
<td>41.3b</td>
<td>7.39b</td>
<td>42.4b</td>
<td>27.1b</td>
<td>23.7a</td>
<td>25.8b</td>
<td>11,329b</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.17</td>
<td>0.012</td>
<td>1.46</td>
<td>0.42</td>
<td>0.36</td>
<td>0.38</td>
<td>5,613</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.001).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.
3Packed cell volume.

Table 4. Processing yields (as a proportion of slaughter weight).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slaughter Weight (g)</th>
<th>Chilled Carcass Weight (g)</th>
<th>Total Breast Weight (g)</th>
<th>Fillets Weight (g)</th>
<th>Tenders Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>4640b</td>
<td>3500b</td>
<td>1198b</td>
<td>987b</td>
<td>216b</td>
</tr>
<tr>
<td>WT</td>
<td>3246b</td>
<td>2373b</td>
<td>744b</td>
<td>611b</td>
<td>129b</td>
</tr>
<tr>
<td>PNT</td>
<td>3664b</td>
<td>2728b</td>
<td>914b</td>
<td>739b</td>
<td>172b</td>
</tr>
<tr>
<td>SEM</td>
<td>30.5</td>
<td>24</td>
<td>13.0</td>
<td>10.8</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.001).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.
3Packed cell volume.

Table 5. Processing yields (as a proportion of slaughter weight).

<table>
<thead>
<tr>
<th>Abdominal Fat</th>
<th>Wings</th>
<th>Leg Quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>Yield (%)</td>
<td>Weight (g)</td>
</tr>
<tr>
<td>NT</td>
<td>69a</td>
<td>1.48a</td>
</tr>
<tr>
<td>WT</td>
<td>49b</td>
<td>1.51b</td>
</tr>
<tr>
<td>PNT</td>
<td>31b</td>
<td>0.80b</td>
</tr>
<tr>
<td>SEM</td>
<td>2.1</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.001).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.

Table 6. Breast meat quality attributes and myopathies.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Meat Color</th>
<th>pH₃₀</th>
<th>Drip Loss (%)</th>
<th>Breast Myopathies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L⁺</td>
<td>a⁺</td>
<td>b⁺</td>
<td>Severe White Stripping</td>
</tr>
<tr>
<td>NT</td>
<td>56.0b</td>
<td>9.6</td>
<td>11.0b</td>
<td>6.37</td>
</tr>
<tr>
<td>WT</td>
<td>59.4b</td>
<td>9.1</td>
<td>10.7b</td>
<td>6.30</td>
</tr>
<tr>
<td>PNT</td>
<td>52.8b</td>
<td>9.0</td>
<td>8.8b</td>
<td>6.31</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2</td>
<td>0.09</td>
<td>0.11</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (P<0.05).

1NT= Neutral Temperature; WT= Warm Temperature; PNT= Pair-fed to WT under NT.
2Pooled standard error of the mean.
Risk assessment toward safe poultry meat

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Abstract

Foodborne diseases remain a persistent threat to human and animal health. Although the sources of infection are mostly unknown, poultry products have repeatedly been implicated as the main sources and the loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products remain a major challenge for the poultry industry worldwide.

Many reports during recent years have shown that Campylobacter spp. and Salmonella are the most common causes of human foodborne bacterial diseases linked to poultry. In addition, several other foodborne bacteria can also enter the human food chain via contaminated poultry carcasses. Furthermore, the development of antibiotic resistance in bacteria, which are common in both animals and humans, are also an emerging public health hazard.

Introduction

In spite of significant improvements in technology and hygienic practices in developed countries at all stages of poultry production, accompanied by advanced improvement in public sanitation, foodborne diseases remain a persistent threat to human and animal health. Food safety and quality are still big issues of major concern in developed countries. In developing countries, the need to produce sufficient food to meet the requirements of population increases, accompanied by bad economic situations often overshadow the need to ensure safe food products. Regardless of this fact, safe food is a fundamental requirement for all consumers, rich or poor.

Many reports during recent years have shown that Campylobacter spp. and Salmonella are the most common causes of human foodborne bacterial diseases linked to poultry. In some areas also verotoxinx producing Escherichia coli 0157:H7 (VTEC), Listeria and Yersinia have surfaced as additional foodborne pathogens causing human illness. Several other toxicogenic bacterial pathogens, such as Staphylococcus aureus, Clostridium perfringens, and Bacillus cereus can also enter the human food chain via contaminated poultry carcasses. In addition, the development of antibiotic resistance in bacteria, which are common in both animals and humans, such as Methicillin Resistant Staphylococcus aureus (MRSA) and Extended-spectrum beta-lactamase (ESBL) bacteria, are also an emerging public health hazard (Hafez and EL-Adawy, 2019).

Campylobacter

Campylobacteriosis is the most commonly reported gastrointestinal disease in the EU. In 2017, 29 EU/EEA countries reported 250161 confirmed cases. The number of reported deaths attributed to Campylobacteriosis increased from 62 in 2016 to 72 in 2017. The infections in humans are mainly transmitted via contaminated food. Campylobacteriosis shows clear seasonality, with a sharp peak of cases in the beginning of the year (Hafez et al., 2015). Campylobacteriosis is frequently encountered in poultry flocks. C. jejuni is responsible for more than 90 % of cases of Campylobacteriosis in humans followed by C. coli. C. lari seems to be more associated with gulls and C. upsaliensis with dogs, but both are regarded as primary pathogens in humans.

In chickens and turkeys Campylobacter spp. are considered part of the normal intestinal flora and Campylobacter infections in poultry are mostly asymptomatic. It is not uncommon to detect several Campylobacter genotypes in the same flock, at the same time or at different ages. Also, often the same strains are encountered in several houses on the farm or in consecutive flocks. Both facts indicate the relative ease of horizontal introduction of Campylobacter into flocks as well as the existence of reservoirs on the farm or nearby (Sahin et al., 2015). After oral infection, Campylobacter colonize the intestines, most importantly caeca and colon. Usually shedding in the faeces, where their concentration can reach up to 10^8 CFU/g. The colonized birds can be a realistic source of infection for the individual bird can quickly become infected with another genotype. In tendency, younger birds are more often infected with C. jejuni, which has the ability to spread more quickly within a flock, while older birds are more often infected with C. coli, which colonizes the birds for a longer time (El-Adawy et al., 2012). Specific flocks that become infected show rapid rate of intra-house transmission and a high isolation rate from caecal swabs, water and litter. (Sibanda et al., 2018).

Since farm visitors and the presence of other animals on the farm are the major risk factors for introduction of Campylobacter to a farm, prevention by improving biosecurity should be an emphasis on these aspects. Fly screens were also highly efficient to decrease the prevalence of Campylobacter-positive flocks. Water pipes and drinkers need to be kept clean. Chlorinating the drinking water or the addition of organic acids as well as organic acids in the feed can reduce colonization of the birds by Campylobacter (Chaveurach et al., 2004; Heres et al., 2004). The use of competitive exclusion or probiotics has yielded inconsistent results (Sahin et al., 2015). Dry bedding in poultry houses will help to limit the spread of the bacteria within a flock. Feed, good gut health, litter material, as well as heating and ventilation in turn are factors to keep litter drier. Additionally under experimental conditions acidifying the litter with aluminum sulfate or sodium bisulfite reduced Campylobacter colonization frequency and populations in the caeca, but the effect wears off as the pH in the litter increases again (Line and Bailey, 2006). Controlling these foods borne organisms requires beside legislations a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism.

There are no commercial vaccines available. Experimentally, attenuated C. jejuni strains used as vaccine did not colonize the birds long enough to induce protection. Vaccination with recombiant Salmonella expressing Campylobacter antigen on the other hand can reduce colonization (Sahin et al., 2015).

Finally, treatment of birds with bacteriophages against Campylobacter can reduce Campylobacter counts in the intestines. However, even in some mixture in time, but several phages are used, resistance rates of the bacteria are high and resistances quickly develop (Fischer et al., 2013, Hauck and Hafez, 2016).

Salmonella

Salmonellosis is the second most commonly reported gastrointestinal infection and an important cause of foodborne outbreaks in the EU (Hafez et al., 2019). In 2016, 95326 laboratory-confirmed cases were reported out of which 134 were fatal. Salmonellosis notification rates have stabilised in the last five years after a long period that was marked by a declining trend. The reported case rate was highest in young children 0–4 years with 89.9 cases per 100 000 population, seven times higher than in adults 25–64 years (ECDC, 2019b). Eggs and egg products continued to be the most commonly identified vehicles in these outbreaks and were also the source in the largest multi-country outbreak linked to eggs from Poland. As stated by EFSA, premature relaxation of effective control measures implemented to date in laying hen farms, in particular the implementation of vaccination programmes and the application of strict hygiene controls, should be avoided (ECDC, 2019b). In fresh broiler meat Salmonella was detected in 6,39% of the 25276 units tested in 2015, and these results were comparable with results in 2015. Testing of 4250 units of fresh turkey meat 7,74% were positive for Salmonella. This was higher than in 2016 (ECDC, 2019b). More details about salmonella and control were published by Hafez (2010).

Antibiotic resistant

The development of antibiotic resistance in bacteria, which are common in both animals and humans, is an emerging public health issue. Controlling these foodborne organisms requires a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism. In addition, the development of novel antibiotics does not keep step with the emergence of antimicrobial resistance in bacteria (Garcia-Rey, 2010).

El-Adawy et al. (2012) investigated 76 C. jejuni isolates were recovered from 67 epidemiologically unrelated meat turkey flocks in different regions of Germany in 2010 and 2011. Only one isolate was sensitive to all tested antibiotics. The numbers of isolates that were resistant to ampicillin, streptomycin, erythromycin, neomycin, amoxicillin and sulphonamides were 69 (80.8%), 61 (80.2%), 58 (76.4%), and 44 (57.9%), respectively. The emergence of a high resistance rate and multidrug resistance to three or more classes of antimicrobial agents was observed. The resistance against sulphamethoxazole/trimethoprim, metronidazole, ciprofloxacin, naladixic acid, and tetracycline was 58 (76.3%), 58 (76.3%), 53 (69.7%), 51 (67.1%), and 42 (55.3%), respectively. Multidrug resistance to three or more classes of antimicrobial agents was found and ranged from 3.9% to 40.8%. Similar results were also found by examination of isolates collected from different free-range turkey flocks in Germany (El-Adawy et al., 2015).

Richter et al. (2012) investigated the prevalence of Livestock-associated methicillin-resistantStaphylococcus aureus (LA-MRSA) in fattening turkeys and people living on farms that house fattening turkeys. Eighteen (90%) of 20 investigated flocks were positive for MRSA. All female flocks were positive, while 8 male flocks were positive. On 12 of the farms (33.3%) of 59 persons sampled were positive for MRSA. None of them showed clinical symptoms indicative of an MRSA infection. People with frequent access to the stables were more likely to be positive for MRSA.

General measures to reduce the incidences foodborne pathogens

The fact that processing plants have not been able to effectively reduce the pathogenic bacteria in poultry products, means that every effort must be made to reduce the incidence of foodborne pathogens and contamination of the live birds before dispatch to processing plants. In general, the major strategies to control food borne infections in poultry should include beside the current legislations, monitoring for microbes (Microbiological risk assessment, MRA), cleaning the production chain from the top especially of vertically transmitted microorganismsuch as Salmonella and limiting introduction and spread of infections at the farm level through Good Animal Husbandry Practices (GAHPs). To achieve GAHPs, effective hygiene measures for poultry houses, environment and the feed. hygiene should be applied. An intensive and sustained rodent control is essential and needs to be well planned and routinely performed and its effectiveness should be monitored. Household pets also constitute a serious hazard. Buildings therefore should be pet proof. In addition, reducing bacterial colonization by using feed additives, competitive exclusion or use of vaccines are further possibilities. Furthermore, hygienic catching and transport to slaughter house should be included.

In aim to reduce the further contamination during slaughter and processing, the slaughter houses should have to adapt dry cleaning regimen. That mean slaughter non-infected flocks firstly and infected flocks at the end of the day followed by cleaning and disinfection of the plant and equipment are essential for maintaining sanitary condition required to reduce contamination. Machine design must permit easy and thorough cleaning. In all cases agent surveillance and monitoring programmes must be adapted and followed strictly in aim to allow early intervention.

Since the success of any safe food depends on personal sanitation, it is essential to incorporate education programs by all people involved throughout the poultry production chain. Also in concept from farm-to-fork, post processing food handling is very important.
References


Impact of increasing incidence of meat quality aberrations on poultry processing

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Abstract

Nowadays most of the world’s chicken meat production is merely based on intensive farming of fast-growing hybrids reaching the slaughter weight in a very short time and having high meat yields. The shift from the sale as whole carcass to ready-to-cook and ready-to-eat products has increased the importance of quality traits of raw meat used for further processing. However, this evolution has promoted the occurrence of muscular abnormalities leading to high downgrading rates and negatively affecting the processing yield and quality traits of ready-to-eat and ready-to-cook products. The poultry industry is facing the occurrence of muscle exhibiting abnormal colour (i.e. PSE- and DFD-like conditions) and concurrently the increasing incidence of growth-related abnormalities involving the myodegeneration of breast fillets (white stripping, woody breast and spaghetti meat). Currently, severely affected meats are downgraded because of their unacceptable visual appearance and diverted for further processing. However, remarkable economic losses and higher processing cost have led to the development of automated systems aimed at discriminating abnormal meat directly in-line. Moreover, when used for further processing, abnormal meats exhibit impaired water-holding and water-binding capacities due to a reduction of both protein content and functionality. In addition, also textural properties of processed products can be affected by the inclusion of abnormal meats. Although a significant reduction of the quality traits can arise when abnormal meats are included in the formulation of grounded and finely comminuted products, proper strategies could be implemented to mitigate the undesirable effects on the technological properties of processed products.

Keywords: Chicken meat, quality, abnormalities, appearance, tenderness.

Introduction

The development in industrialization and specialization of broiler meat production chains that took place starting from the end of World War II, has led to a remarkable increase in both efficiency and chicken meat production worldwide. In recent years, the lifestyle changes have also dramatically modified the way in which the poultry meat is marketed and consumed. Therefore, food technologies have become part of the poultry industry and today much of the production is marketed in the form of cut-ups and processed products (Table 1).

As a result, nowadays, most of the World’s production is merely based on intensive farming of few fast-growing hybrids rapidly reaching the slaughter weight and having high meat yields. In addition, because of consumers’ preference for breast meat along with the expanding market of cut-ups and processed products, broilers are usually slaughtered at increased weights. Within this context, as a result of the shift in market form from whole carcass to ready-to-eat and ready-to-cook products, the importance of quality traits of meat used for both fresh consumption and in the formulation of processed products has remarkably increased. This evolution has led to extreme modifications in the modern hybrids which are currently selected and used worldwide to produce chicken meat. Nonetheless, the differences existing in meat quality among the most popular hybrids (i.e. Ross, Cobb and Hubbard) are very limited if compared to the ones observed among and within the medium- and slow-growing genotypes. Thus, the changes in meat quality traits existing in different fast-growing hybrids mainly arise by farming factors and, especially in recent years, by the pre-slaughter and slaughtering phases. In this regard, it is also well known that some features observed in fast-growing hybrids (i.e. muscle hypertrophy, accentuation of glycolytic metabolism of the muscles, poor thermoregulatory capacity, skeletal and vascular fragility, insufficient vascularisation), might directly or indirectly be induced by selection, predisposing the occurrence of meat abnormalities with an increased incidence within the past 30 years (Petracci et al., 2015; Velleman, 2015).

Finally, legislations, on its own, can never be sufficient to ensure the production of safe food. Rather, the industry itself, from producer to retailer, has a responsibility to ensure the safety of their products.
Emerging abnormalities as related to myodegeneration

In recent years, a new group of muscle abnormalities characterized by myodegeneration has appeared. This group includes manifestations of white striations parallel to muscle fibres mainly occurring on the ventral surface of breast fillets (white striping, WS; Kuttappan et al., 2009), woody breast condition (WB), often associated with white striping, where muscles are palpable hard, out bulging and pale (Silvio et al., 2014) and “spaghetti meat” abnormality (SM), distinguished by the tendency toward separation of muscle fibre bundles (Baldi et al., 2018).

Nowadays, the occurrence of abnormalities affecting the pectoral muscle (i.e. WS, WB and SM), which is the most valuable part of broiler carcass, causes increased downgrading of meat because of the impaired visual appearance, sensory and technological qualities (when used for further processing) of meat, with a special reference to woody breast abnormality (Petracci et al., 2019).

These abnormalities appear only in fast-growing broiler hybrids, so it is therefore clear that pectoral muscle hypertrophy and high growth rate could be cited as the promoting causes (Petracci et al., 2015).

Regarding meat quality traits, altered colour and ultimate pH values were observed within the P. major muscles affected by abnormalities. If compared to their unaffected counterpart, the affected cases revealed a remarkably higher ultimate pH values, associated with a lower glycogen content (Berti et al., 2007; Mutryn et al., 2015). Indeed, even if it was reasonable to hypothesise that microbial shelf-life of meat affected by muscle abnormalities can be remarkably reduced as a consequence of their ultimate pH values, recently it was surprisingly demonstrated that microbial shelf life is shorter in normal than in abnormal meat (Gratta et al., 2019). On the other hand, reduced water holding and water binding capacities associated with the occurrence of muscle abnormalities and likely linked to an overall reduction in protein functionality, with more pronounced effect being exerted by the woody breast and spaghetti meat rather than the white striping defect (Petracci et al., 2019). This phenomenon might be partly due to protein aggregation and cross-linking following oxidation (Soglia et al., 2016; Baldi et al., 2019) and to the overall substantial reduction and altered profile of muscular contractile and sarcoplasmic proteins typically observed within the abnormal muscle tissues (Baldi et al., 2018). Dealing with that, the overall impairment of the water holding capacity of meat was corroborated by nuclear magnetic resonance (NMR) relaxation properties examining both the relative intensity and the T2 transverse relaxation time for the three proton populations (bound, intra- and extra-myofibrillar water) identified within the muscle tissue. According to our previous study, the remarkable increased proportion and mobility of the extra-myofibrillar water fraction (the potential drip of the meat) may account for the lower water holding ability of the abnormal cases during processing and storage (Soglia et al., 2016; Baldi et al., 2019; Zimermann et al., 2017; Zimermann et al., 2017; Zimermann et al., 2017). The evolution of muscle fibres not only affects the muscle appearance but also significantly affects visual appearance and diverting for further processing (Kuttappan et al., 2016; Petracci et al., 2017; Zimmermann et al., 2012). However, there are margins to mitigate undesirable effects on technological properties of processed products when abnormal meats are included in the formulation of ground and finely comminuted meat products, albeit significant quality reduction can arise when high-quality processed products (i.e. enhanced whole-muscle and ground products) are manufactured by using especially WB abnormal raw meat (Bowker et al., 2018; Brambilla et al., 2018; Maxwell et al., 2018). In addition, latest studies highlighted that overall manifestation of muscle abnormalities mainly affects the superficial section of P. major muscle, while the deep section is barely affected (Bowker & Zhuang, 2016; Baldi et al., 2018; Bowker et al., 2018). Therefore, one possible strategy to limit meat downgrading could be to separately process the superficial (ventral) and deep (dorsal) layer of the abnormal pectoral muscle for exploiting their distinctive traits (Baldi et al., 2019; Bowker et al., 2018). Table 2 presents processing solutions for alleviating meat quality consequences of occurrence of white striping (WS), woody breast (WB) and spaghetti meat (SM) (modified from Petracci et al., 2019).

Table 2. Processing solutions for alleviating meat quality consequences of occurrence of white striping (WS), woody breast (WB) and spaghetti meat (SM) (modified from Petracci et al., 2019).

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Meat processing solutions</th>
<th>Effectiveness</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>Breast fillet portioning (separation of dorsal and ventral portions)</td>
<td>high</td>
<td>Bowker &amp; Zhuang (2016)</td>
</tr>
<tr>
<td>WS/SM</td>
<td>Vacuum tumbling (whole-muscle)</td>
<td>poor</td>
<td>Soglia et al. (2016)</td>
</tr>
<tr>
<td>WB</td>
<td>Coarsely mincing (cooked patties)</td>
<td>high</td>
<td>Brambilla et al. (2017, 2018)</td>
</tr>
<tr>
<td>WB</td>
<td>Finely mincing (meat batters and meat balls)</td>
<td>high</td>
<td>Xing et al. (2017)</td>
</tr>
<tr>
<td>WB</td>
<td>Coarsely mincing (cooked patties)</td>
<td>poor</td>
<td>Madruga et al. (2019)</td>
</tr>
<tr>
<td>WB</td>
<td>Finely mincing (meat batters and meat balls)</td>
<td>poor</td>
<td>Chen et al. (2018)</td>
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Conclusions

The evolution of the chicken meat market has increased the relative importance of quality parameters that affect the appearance (in terms of absence of visual defects and abnormal colours) and the sensory profile with special emphasis to tenderness. However, during the past decades, the genetic characteristics of current fast-growing hybrids have certainly promoted the occurrence of a high number of abnormalities that are increasing the meat downgrading rates for fresh meat retailing and decreasing the nutritional, sensory and technological qualities of raw meat materials used as raw materials in further processing. Therefore, it seems that poultry industry cannot longer postpone a closer view of problems related to the proper muscle growth and the quality of resulting meat when selecting genotypes for broiler production.

References


Introduction

The European Commission is promoting the transition to a circular economy, proposing to make the best use of resources with the aim of reducing environmental pollution and conserving natural resources. One way to develop a circular agri-food system is by using by-products from the food industry to produce animal feed, contributing to obtain more environmentally friendly production systems.

Our research group has participated in different projects (Ref. FP6 FOOD-CT-2004-007020, CYCIT AGL2010-22008-C02, MAGRAMA 20130020000782 and CYCIT AGL2015-64431-C2-1-R) dealing with the characterization of quality and safety of fat-by-products for uses foods, within the integrated approach from farm to fork. Results of the projects indicate that vegetable fat-by-products from chemical and physical refining are economically interesting alternatives for feed and meat producers, if compared with conventional oils (Nuchi et al., 2009; Tres et al., 2012; Rodriguez-Sanchez, 2019, and Vilarrasa et al., 2015a y 2015b).

In this presentation, the most relevant results about the use of fat-by-products in the lipid composition of broiler meat are presented, which will lead us to the final remarks.

Fat by-products

Acid oils (acid oils from chemical refining and fatty acid distillates from physical refining) and lecinthin are by-products obtained from the refining process of crude native oils (Catalogue of feed materials; Commission Regulation (EU) No 68/2013) that can be used in broiler chicken diets. They are alternative sources of energy and they have a similar fatty acid (FA) profile to their corresponding crude oils, but different molecular structures. The economic viability of these by-products will depend on the price differential, with respect to their corresponding crude oil. The aim of the refining process is to reduce FFA content and to remove other undesirable compounds. Chemical refining (also known as alkali refining) is the most used technique for the refining of many seed vegetable oils such as soybean oil. Crude lecithin is obtained during the first step of refining (de-gumming step) and consists of a lipid mixture mainly composed of polar lipids (> 60%), in particular phospholipids (PL). It is important to pay attention to the repercussion of the use of fat by-products, not only on the broiler nutritive utilization but also on the quality of the meat. This paper presents interesting results about the effect of the dietary supplementation with acid oils, lecinthin and other fat by-products on the lipid quality of broiler meat. Our aim is to establish how the different molecular structure (TAG, FFA and PL composition) and nutritional properties of these alternative feeding fats affect the lipid composition of broiler meat in terms of FA profile, TAG structure and oxidative stability.

Keywords: acid oils, soybean lecithin, re-esterified oils, broiler-meat lipid quality

Acid oil (rich in FFA) can be used for chemical re-esterification with glycerol (a by-product from biodiesel industries) to form new acylglycerols. Depending on the process conditions, MAG and DAG in the re-esterified oil can be at higher amounts than in a crude oil, which can boost the processes of fat digestion and absorption due to their lower melting point. Also, when randomized esterification is used, the proportion of saturated fatty acids (SFA) located at the acylglycerol sn-2 position can be increased, with respect to the corresponding crude oil. While from a production point of view this can be interesting to increase digestibility and productive parameters, mainly in oils rich in long-chain SFA, it has to be verified that it does not entail an increase in meat SFA, since this would not be nutritionally interesting.

During the last several years, the global aim of our research has been to characterize these new fat sources, to investigate the potential use of these oils (also known as dry or steam refining). This process has been carried out with the hypothesis that those fat co- and by-products (that meet critical control parameters for animal feed, such as maximum levels of moisture, impurities and unsaponifiables (MIU), trans fatty acids (t-FA) and lipid oxidation compounds) could provide valuable nutritional characteristics in feeding animals without any health risk while ensuring good quality and safe meat (Tres et al., 2012; Vilarrasa et al., 2015a, 2015b; Rodrigo-Sanchez et al., 2019; Vinado et al., 2019). The results regarding the effect of the supplementation of lecinthin (crude soybean lecithin), acid oils (soybean oil from chemical refining and palm fatty-acid distillate from physical refining) and randomly re-esterified oils on the lipid composition of broiler chicken meat in terms of FA profile, TAG structure and oxidative stability are given below.

Effect of dietary fat by-products on broiler meat quality

Crude Soybean Lecithin

Soybean lecithin is a co-product extracted during the process of oil refining that may represent an economic alternative energy source for broiler feeding. Its high content of PL (about 60%) represents an added value as an emulsifier and may improve fat absorption. The objective of this study is to evaluate the inclusion of crude soybean lecithin (L) as dietary-added fat in broiler diets, replacing soybean oil (S) as the energy source, and to study its influence on productive performance, FA digestibility, feed apparent metabolizable energy and the FA profile of abdominal fat (A) and meat. For this, a control basal diet was supplemented at 3% with S50 (S:50%), 10% with S50 (S:50%) and 30% with S50 (S:50%) and increased amounts of L (1%, 3% and 5%). These treatments were randomly assigned to one of four experimental treatments (six replicates/treatment). Replacement of S by L did not result in significant differences on AF deposition among experimental treatments (P > 0.05). Regarding the composition in FA, the FA profile of the AF was similar to the FA profile of the meat. The FA profile of the meat (thigh with skin) reflected the FA profile of the diet, and according to most of the published data (Barroeta, 2017; Barroeta et al., 2009; Ferrini et al., 2008; Vilarrasa et al., 2015a and 2015b). Total replacement of S by L increased monounsaturated FA (MUFA) and SFA content, in particular palmitic acid, whereas it reduced polyunsaturated FA (PUFA) content (P < 0.01). These results demonstrated that the FA profile of the meat and AF was more influenced by dietary composition of FA (saturated degree) rather than by their supply as PL or TAG.

Results of this experiment lead to conclude that the inclusion of L, as a partial replacer of S up to 2%, is suitable in grower-finisher diets, maintaining similar performance, FA and energy utilization results in small changes in abdominal adipose tissue and meat FA composition, ensuring the quality of the final product.

Acid oils

Different studies were carried out to determine the potential use of acid oils in broiler chicken diets. The aim was to assess the effect of the dietary FA level with a different saturation degree of dietary fat on productive performance, FA digestibility and lipid composition of the meat in broiler chickens. A wheat-and-soybean-meal-based diet was supplemented at 6% with different oil sources. Eight experimental diets were obtained replacing crude soybean oil by soybean acid oil from chemical refining, or crude palm oil by palm fatty-acid distillate from physical refining (PPAD). Thus, there were four soybean oils and four palm oil (P, saturated) diets with four increasing levels of dietary FFA (5%, 15%, 35% and 50%). Regarding the FA profile (%) of the meat (thigh with skin), unsaturated S diets resulted in higher PUFA (S33.5% vs P14.5%) and lower MUFA (S38.5% vs P52.1%) and SFA (S28.0% vs P33.2%) levels than in P diets (P< 0.01). On the other hand, the effect of the dietary FA content related to the dietary saturation degree. In S diets, as the dietary FFA level increased, dietary SFA content increased. Consequently, the meat of the animal fed the highest FFA level showed a higher SFA (S5.25% vs S5.30%) and MUFA content (S3.70% vs S5.43%), and a lower PUFA content (S5.36% vs S5.25%) than those fed S (P < 0.01). The FA profile remained more constant, thus the increase in the dietary FA content did not change the FA profile of the meat. (MUFA 51.4-55.3; PUFA 14.7-14.1; SFA 33.8 - 32.4; MUFA: 51.4-55.3; PUFA: 14.7-14.1). The results allow us to conclude that the dietary FA content has a greater impact on FA composition of the chicken meat than the dietary FFA level, which is in accordance with Vilarrasa et al. (2015a). The inclusion of acid oils (acid soybean oil and palm fatty-acid distillate in grower-finisher broiler chicken diets could partially replace the corresponding crude oils, according to FA absorption and performance results (Rodrigo-Sanchez et al., 2019).

In addition to the FA profile, other aspects that might influence meat quality include the presence of t-FA and oxidative stability.

- FA intake has been associated with an increased risk for coronary heart disease; therefore, it is important to control its content in poultry meat. When 6% of PFAD was included in the diet (4.4 g/kg t-FA), the chicken meat presented 24.1mg/100g t-FA (Tres et al., 2012), although the addition of non-hydrogenated PFAD in chicken feed leads to meats with low levels of t-FA. Indeed, 100g


References

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Abstract

The use of fats as feed ingredients is a widespread practice in order to enhance the energetic value of the diet and provide some specific and essential nutrients for poultry. Crude fats and oils are mainly constituted by triacylglycerols (TAG > 90%), and their calorific value is about 9.4 kcal/g. There is an increasing interest about searching for alternative fat sources at competitive prices, for feed formulation. Some of them are fat co- and by-products coming from edible oil refining. They have a substantial energetic value and constitute a residual product to be recycled in order to improve sustainability of the food chain. Among them, the most relevant ones are acid oils (acid oils coming from chemical refining and fatty acid distillates from physical refining) and crude lecinthin. Acid oils are rich in free fatty acids (FFA) and have a similar fatty acid (FA) composition to their respective crude oils. Crude soybean lecithin is obtained during the de-gumming step and mainly consists of a mixture of polar lipids (> 60%), particularly phospholipids (PL). It is important to pay attention to the repercussion of the use of fat-by-products, not only on the broiler nutritive utilization but also on the quality of the meat. This paper presents interesting results about the effect of the dietary supplementation with acid oils, lecinthin and other fat by-products on the lipid quality of broiler meat. Our aim is to establish how the different molecular structure (TAG, FFA and PL composition) and nutritional properties of these alternative feeding fats affect the lipid composition of broiler meat in terms of FA profile, TAG structure and oxidative stability.

Keywords: acid oils, soybean lecithin, re-esterified oils, broiler-meat lipid quality

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of this meat corresponds to less than 0.0001% TFA of the daily energy intake of 2,400 kcal, which is far below the values reported to increase cardiovascular risk.

The oxidative stability and shelf life of meat depend on the balance between certain FA (such as PUFA), pro-oxidants and antioxidants. Oxidation of PUFA leads to primary and secondary oxidation products. Several of them might cause off-flavors in meat, such as rancid flavors. Also, a range of biological effects, which are mainly detrimental and related to certain chronic and degenerative diseases, has been described for some of them. The aim of this study is to investigate the potential use of acid oils, rich in FFA, in comparison with their corresponding crude oils in broiler chicken diets, which are mainly detrimental and related to certain chronic and degenerative diseases.

The oxidative stability and shelf life of meat depend on the balance between certain FA (such as PUFA), pro-oxidants and antioxidants. Oxidation of PUFA leads to primary and secondary oxidation products. The dietary treatments were the result of including FFA of one of the following experimental fats to the basal diet: crude palm oil (P), PEAD (PA), crude soybean oil (S) and soybean acid oil from chemical refining (SA). Results have demonstrated that oxidative stability of fresh meat depends on the FA composition, not by the dietary FFA content. The susceptibility of fresh meat to oxidation depends on the FA profile, not on their FA composition, not by the dietary FFA content. The susceptibility of fresh meat to oxidation depends on the FA profile, not on their FA composition.

The increased sn-2 SFA content (14.3%) or SA (14.1%), and higher in PE (28.1%) than in P (23.1%) or PA (21.0%). This pattern was also found in meat (thigh plus skin) for the FA at the sn-2 position, which was higher in SE (14.3%) than in P (14.1%), and higher in PE (28.1%) than in P (21.0%).

The increased sn-2 SFA content, together with the increased MAG and DAG content of re-esterified oils, could exert a boosting effect on the SFA apparent absorption that could be related to the higher SFA content and higher SFA at the sn-2 position of the meat of broilers fed re-esterified oils. However, these observed increases in SFA are very small and may not actually be biologically or nutritionally relevant from the human consumption point of view.

Thus, results of this study suggest that the use of acid or re-esterified oils may potentially be used as alternative fat sources for the feeding of chickens without significant harmful effects or essential changes in the lipid characteristics of the meat, and showing similar or even higher total FA apparent absorption results than their corresponding crude and acid oils.

In general, the fat saturation degree exerted a greater impact on FA apparent absorption, growth performance, carcass fat depots and FA composition of abdominal adipose tissue and meat than did the fat molecular structure.

Taken altogether, the results of these studies demonstrate that fat co- and by-products (soybean lecithin, acid oils and re-esterified oils), which meet established critical control parameters for animal feed, are good, alternative fat sources to be used in broiler chicken grower-finisher diets, without impairing performance, FA digestibility and causing minor changes in the lipid characteristics of the meat. Therefore, the inclusion of by-products from the oil refining process, in animal feed, is a good strategy that allows for an adequate production and suitable quality levels of the final product.

References


Characterization of single-nucleotide GDF8, WWP1 and PPARGC1A polymorphisms genes affecting skeletal muscle growth and energy metabolism in broiler chickens

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Advances in genetic selection, farming practices and nutrition, the production of broiler chickens has become more efficient. Genetic selection has contributed significantly to the improvement in growth rate, biological efficiency, breast yield, longevity, and leg health. In animal breeding, meat quality is a complex trait, referring to the compositional, visual, and sensory traits of carcass, or its retail cuts. It has been well documented that myofibertype composition can profoundly influence postnatal growth and meat quality. Breast muscle myopathies (BMM) can be caused by aberration in genes affecting both growth and energy metabolism of skeletal muscle cells. In the present work we investigated three genes, Growth differentiation factor-8 (GDF8) and E3 ubiquitin-protein ligase (WWP1), involved in skeletal muscle growth, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), that plays a role in glucose and fatty acid metabolism. For the experiment 90 chickens of six different genetic lines were recruited. Post mortem breast fillets were scored for different degrees of white stripping and fatty acids were measured both in breasts and legs; DNA was extracted from breast meat. The III and VIII exons for PPARGC1A, I and III exons for GDF8, X exon for WWP1 were amplified by polymerase chain reaction (PCR) by using primers designed in the flanking introns and then sequenced. Four SNPs, that caused synonymous mutation, were detected in GDF8. Fifteen SNPs were identified in intron region of WWP1. Polymorphism identified by Imamura et al. (2015) in WWP1 X exon associated with muscular dystrophy, was not present in these chicken lines. For PPARGC1A four single nucleotide polymorphisms (SNPs), were identified, three in the flanking region of III exon, that didn’t influence splicing sites and three in VIII exon, that caused following missense mutation: C348W, R456Q, E458T. Allelic, genotypic and haplotypic frequencies were calculated for all SNPs. The effects of the non-synonymous variantt on PPARGC1A protein function were evaluated by using the SIFT (Sorting Intolerant From Tolerant) algorithm and the substitution both of a Cys with a Trp and a Glu with a Thr in the C-terminal region of protein was predicted not tolerated. Because proteins encoded by the three investigated genes are in the same metabolic network and have been shown to interact, the identified mutation could have an effect on skeletal muscle structure and growth.

Keywords: muscle, energy metabolism, GDF8, WWP1, PPARGC1A

The Relationship Between Metatarsus (Shank) and Some Body Characteristics in Broiler Pure Line and Cross Genotypes

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Abstract
In this study, the relationship between foot and body defects and the traits of metatarsus (shank) bone have been demonstrated some of the growth traits in broiler pure-lines. The study material consisted of two male pure-lines (B1, B2), three female pure-lines (A1, A2, A3) and six cross genotypes (B1xA1, B1xA2, B1xA3, B2xA1, B2xA2, B2xA3) produced by their two-way crossing. 10 males and 10 females were slaughtered in each group at the 6th week. Body weight (CA), breast weight (GA), thigh weight (BA), and the foot pad dermatitis levels, foot-finger disorders and breast defects were determined in 940 broilers at 42nd day. Metatarsus features were determined after cleaning and drying of the feet cut from the tarsal joint. Traits such as metatarsus weight (MA), length (MU), shear strength (KM), MA/CA, MA/BA were evaluated. Differences between the groups were found significant (P<0.05) in all traits except the shear strength, while male broilers had higher values in all traits. Some of the metatarsus traits of the male lines have also been observed in cross groups. It is predicted that the high correlation between the length, diameter and weight of metatarsus and their relationship with FPD, foot and finger disorders can be used for selection.

Keywords: Metatarsus length, metatarsus diameter, metatarsus strength, broiler pure-lines, cross genotypes
Introduction

In broiler production, the time to reach the slaughtering age was reduced, while the feed conversion ratio was improved and the effective growth rate could be increased (Rekaya et al., 2012). In these developments, classical and molecular selection methods which have been made for the body weight, breast width, different body traits, conformation and feed efficiency have been important contributions for many years (Bessi, 2006; Buzala et al., 2015; Nangsuay et al., 2015). Thus, the level of feed conversion, which was 2.3 in 35-day-old age in 1985, could be reduced to 1.5 in 2010 (Siegel, 2014). 85-90% of the improvements in the performance traits are caused by the selection applied to the live weight and body traits (Havenstein et al., 1994).

Parallel to the fast growth and slaughtering age, some metabolic disorders in the broiler parent and offspring, foot and leg defects, and lesions in the breast and other body regions have emerged. Fast growth and increase in breast ratio cause foot-leg problems (Arthur and Albers, 2003; Whitehead et al., 2003; Knowles et al., 2008); these problems are reported to be slower in slow growing broilers (Shuches and Botelho, 2012). Sarica et al. (2014) found that the development of male lines does not occur on the same level as the increase in body weight causes some discrepancies in the body and decreases in walking ability (Nestor et al., 1985). Foot-leg problems occur under the influence of genetic structure and environmental factors (Yalcin et al., 1996). The prevention of this problem, which has caused economic issues and animal welfare, has become an important criterion in selection programs (Akbas et al., 2013). The length, diameter and quality of the Shank (metatarsus) used as a selection criterion in broiler vary depending on gender (Leeson and Caston, 1993), genotype, live weight, feeding (Romero-Sanchez et al., 2007) and diseases (Whitehead, 2007). The properties of the metatarsus bone are determined by parameters such as breaking strength (Park et al., 2003; Kim et al., 2006), density, mineral level (Onganyo et al., 2003) and ash level (Park et al., 2003; Shim et al., 2008). It was reported that the length of metatarsus bone in fast and slow growing parents (Dudgeon, 2010) was affected also in females (McGary et al., 2013) and its length did not change significantly after 28 weeks of age (Salahi et al., 2014). It has been stated that due to metatarsus length, foot health changes and long metatarsus may be a source of foot problems in fast growing broilers (Gao et al., 2010). It is possible to improve some properties by taking advantage of the relations between metatarsus and other body traits (Rizzi et al., 1994). Although the relationships between body weight, breast weight, and some other morphological features and metatarsus traits were evaluated (Udeh and Ogbu, 2011), their relationship with FPD (foot pad dermatitis) which is a serious problem in broiler parents and their offspring has not been sufficiently demonstrated.

Material and methods

The material of the study consists of 3 pure female lines (A1, A2, A3), 2 pure male lines (B1, B2), and their crosses (B1xA1, B1xA2, B2xA1, B2xA2, B2xA3) used in broiler parent breeding. From 11 different genotypes, the chickens were placed in the pens (18x2.2 m) at 20-24 chickens in 1.5 meters in height with 4 replications. The chickens were reared in the factory, and 4 different feeds were used during the growing period. Chickens were reared 42 days of age and fed on ad libitum basis on 4-phase feeding program (1st: 1-10 day; 2nd: 11-21 day; 3rd: 22-35 day; 4th: 36-42 day). Water was given on ad libitum.

At the end of the study (day 42), live weight of all chickens, foot pad dermatitis scores and foot and finger problems were determined (Yamak et al., 2015). Finger crookedness; 0: healthy finger structure, 1: 1-2 finger, 2: 3-4 finger, 3: 5-6 finger distortion and 4: 7-8 finger crookedness scoring which uses a scoring scale was used. In foot pathology, foot and finger crookedness, chickens were classified clinically by holding their wings and angle of the leg bone tarsal joint (Letterier and Nys, 1992).

10 male and 10 female chickens were randomly slaughtered from each genotype group and the left metatarsus were marked according to their genotype and sex. Bones were placed in the freezer for laboratory analysis. Then, the bone was kept in water for 96 hours. After that they were ground in a commercial feed factory, and the different feeds were used during the growing period. Chickens were reared 42 days of age and fed on ad libitum basis on 4-phase feeding program (1st: 1-10 day; 2nd: 11-21 day; 3rd: 22-35 day; 4th: 36-42 day). Water was given on ad libitum.

Table 1 Metatarsus and changes in some body traits in genotype groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FPD score</th>
<th>Foot score</th>
<th>Finger score</th>
<th>Breast score</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM (♂)</td>
<td>0.128ab</td>
<td>0.123b</td>
<td>0.121c</td>
<td>0.115a</td>
</tr>
<tr>
<td>BU (♀)</td>
<td>0.128ab</td>
<td>0.123b</td>
<td>0.121c</td>
<td>0.115a</td>
</tr>
</tbody>
</table>

Discussion

As a result, it is predicted that high correlation coefficients between the length and diameter and weight of metatarsus and their relation with FPD, foot and finger disorders can be used in selection. These traits can also be taken in live animals (length and diameter), according to which selection will contribute to other traits.

References


ASABE. Shear and three-point bending test of animal bone. ANSI/ASAE S459, 2012.


BIZERRAY D, LETERRIER C, CONSTANTIN P, PICARD M, FAURE JM. Sequential feeding programs (Akbas et al., 2013) and diseases (Whitehead, 2007). The properties of the metatarsus bone are determined by parameters such as breaking strength (Park et al., 2003; Kim et al., 2006), density, mineral level (Onganyo et al., 2003) and ash level (Park et al., 2003; Shim et al., 2008). It was reported that the length of metatarsus bone in fast and slow growing parents (Dudgeon, 2010) was affected also in females (McGary et al., 2013) and its length did not change significantly after 28 weeks of age (Salahi et al., 2014). It has been stated that due to metatarsus length, foot health changes and long metatarsus may be a source of foot problems in fast growing broilers (Gao et al., 2010). It is possible to improve some properties by taking advantage of the relations between metatarsus and other body traits (Rizzi et al., 1994). Although the relationships between body weight, breast weight, and some other morphological features and metatarsus traits were evaluated (Udeh and Ogbu, 2011), their relationship with FPD (foot pad dermatitis) which is a serious problem in broiler parents and their offspring has not been sufficiently demonstrated.

Table 2 FPD, foot, finger and breast scores in genotype groups

<table>
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<tr>
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References


ASABE. Shear and three-point bending test of animal bone. ANSI/ASAE S459, 2012.


Longitudinal RNA-seq Analysis of Tissue Development Reveals the Hub Genes that Influencing the Chicken Intramuscular Fat and Abdominal Fat Deposition

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“typical” 1957 and 1991 broiler

In the chicken industry, high intramuscular fat (IMF) content contributes to desirable meat quality. Abdominal fat (AF) weight will increase together with the IMF in the chicken body, however, high AF weight will also reduce feed utilization efficiency. The desired breeding goal is the increase of IMF and the decrease of AF at the same time. There is limited knowledge on the relationship between the dynamic status of the gene expression and the fat deposition in chicken. Our goal is to find out the hub genes that related to high IMF content and low AF weight. We performed a longitudinal transcriptome study from breast muscle (BM), AF, and liver tissues in an inbred slow growing meat-type female chickens at 9 developmental stages (day 12, day 17 of the embryonic period, and 1, 7, 21, 56, 98, 140, and 180 days after hatching). The within tissue differentially expressed genes (DEGs), fold change > 1.5 and adj-p < 0.05 between adjacent developmental stages were high between early stages. The number of up- and down-regulated DEGs in D21 vs D56 is the lowest of all comparisons. The DEGs between adjacent stages were enriched in the PPAR signaling pathway, and the fatty acid biosynthesis in all 3 tissues, representative gene patterns were clustered. Fat acid degradation and adipocytekine signaling pathway were enriched (FDR < 0.05). The corresponding correlation between the gene expression and the traits of IMF and AF weight were analyzed by WGCNA. A module of 130 genes significantly positively related to the IMF content was detected in BM (p = 3e-08). Another 2 modules of 83 and 133 genes significantly negatively related to the AF weight were detected in AF (p = 8e-04, p = 1e-05, respectively). Several hub-genes were worked out, such as POMC, IFNAL1, CCRBL, CYPBP1, ENSGALG000000035643 for high IMF content, and MIR106, ENSGALG00000024621, and ENSGALG00000307304 for low AF weight. In conclusion, the comprehensive analyses of RNA-seq characterized the dynamic changes both in expression- and function-level in chicken BM, liver, and AF, at 9 representative development stages. Our findings offered a fresh insight into longitudinal changes of mRNA expression in chicken BM, liver, and AF, and suggested that the changes represent previously unappreciated regulatory nodes that potentially contribute to the further chicken breeding on high IMF content and low AF weight.

Keywords: chicken, intramuscular fat, abdominal fat, RNA-seq

Fatty acid profile in liver of different poultry genetic strains

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The beneficial effect of n-3 Polyunsaturated Fatty Acid (PUFA) on several aspects of human health is well known. However, humans requires a certain amount of these compounds in foods being biosynthetic for precursor (n-Linoleic acid - ALA) and rather inefficient in thePUFA with more than twenty carbon atoms(LCP) synthesis. Fish products represented the main source of n-3 PUFA but are becoming progressively scarce and the ovious of fishing areas worldwide became unsustainable. Therefore, the ability of terrestrial ruminants to elongate and desaturate ALA in LCP meat could be carefully considered to find other observable sources of n-3. The synthesis of n-3 LCP compounds is in competition with the n-6 ones due to the same enzymatic pathway and there are many factors affecting this metabolism. The aim of this study was to investigate the effect of genotype, sex and slaughter age on the LCP synthesis. Different genotypes with different growth rate, Ross (Fast-growing), Leghorn (Slow-growing) and their crossbred (Leghorn X Ross) were reared in the same rearing conditions. Male and female chickens were slaughtered at two different age45 and 81 days) to analyse the liver fatty acid profile with GC-MS. The main compounds evaluated were: Linolenic acid (LA) and ALA, respectively n-6 and n-3 precursors, Arachidonic acid (AA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) respectively, n-6 and n-3 LCP derivatives. The results showed that the presence of LA and ALA on the liver increased with the age in all the genotypes. In particular, females of Leghorn and crossbred showed the higher amount at both age, while in Ross significantly
higher values were observed only at 8 days. Both, MCP-n-3 and n-6 have been influenced by age, sex and genetic strain; the amount of EPA, DHA and AA increased with the age and they were higher in Leghorn and crossbred females. However, the genotypes showed the main effect and the highest amounts of EPA and DHA were detected in Leghorn chickens. According to the resource allocation theory, Slow-growing genotypes use more dietary energy for the metabolism functions respect to the Fast-growing that are oriented to the growth traits, it is known that the synthesis of EPA and DHA is more expensive because need a more complex and costly enzyme pathways (desaturation and elongation). In the same line it should be hypothesised that Fast Growing strain had a higher catabolism of n-3 (beta-oxidation) for energy purpose.

Keywords: PUFA, genotypes, sex, age

Effect of post-hatch holding time and feed access time on live performance and carcass characteristics

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2Petrsime NV, Belgium

This study investigated the effect of post-hatch holding and feed access time on subsequent live performance and carcass characteristics of Mesopotamian broiler chickens. These broilers have been raised in the region of the Mediterranean basin and they are well known for their high quality meat characteristics including wooden breast (WB) and white striping (WS). The birds will have different ages and sex, and their weight will be recorded at different times. The results will be used to improve the feed conversion ratio (FCR) and mortality at 35 days of age, early feed access (2h) tended to have higher FCR than other groups. No significant differences among groups were observed on the percentage of wings, WS, or WB score (P>0.05), but 2h group had a significantly higher PG (P<0.05). When birds were weighed at the end of the experiment, there was no significant difference in FCR, but 2h group had a significantly lower BW and food consumption (P<0.05). Although there was no significant difference in carcass yield, WS, and WB score, 2h group had a significantly higher PG (P<0.05), but 36th group had a significantly lower BW and food consumption (P<0.05).

Keywords: feed access time, post-hatch holding, BW, mortality, breast muscle myopathies

The Effects of Monochromatic Lighting with Different Wavelengths on Carcass Characteristics and Meat Quality of Broiler Chickens

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2Namik Kemal University, Faculty of Agriculture, Animal Science Department Tekiridağ, Turkey

The lighting is a determinant environmental factor to influence the performance, health and behavior characteristics of birds. In the past, incandescent lighting was used mostly in poultry houses, but recently LED lighting is being used due to possible advantages in broiler performance under lighting by various colored LED lights. The aim of this study is to determine the effects of monochromatic lighting with different wavelengths on carcass characteristics and meat quality of broiler chickens raised under extensive indoor system. A total of two hundred broiler chickens were separated to four experimental groups and placed into four homogenous floor pens. A light intensity of 45 lux at the level of bird was achieved with four environment includes white for control, red, green and blue LED lighting (660 nm, 560 nm, and 480 nm, respectively) for treatment groups. According to the results of the study, in terms of slaughter weight and breast weight, the blue LED group was found higher than other experimental groups (P<0.05). The green LED group also had a higher average in terms of carcass yield (P<0.05). On the other hand, there were no statistically significant differences between the mean values of experimental groups in terms of other carcass traits, meat color, ultimate pH, water holding capacity, shear force. In conclusion, it is possible to say that the blue and green LED lighting has the favorable effects on body weight and some carcass characteristics of broiler chickens.

Keywords: Monochromatic lighting, Broiler, Carcass, Extensive indoor, Meat quality

Effect of yellow lupine seeds (Lupinus luteus L.) and silkworm pupae (Bombyx mori) pupae meal as alternative protein source in broiler chicken diets on growth performance, carcass parameters and meat quality

Sylwia Anna Grzewska Dudek, Mariusz Pietras

National Research Institute of Animal Production, Department of Nutrition Physiology

The aim of the study was to determine the effect of yellow lupine seeds and silkworm pupae meal as a protein source instead of soybean meal in diets for broiler chickens on performance indices, carcass parameters, chemical composition and sensory quality of the meat. The experiment was carried out on 120 28-day-old Ross 308 broilers. At the age of 21 days the birds were randomly assigned to three experimental feeding groups. Chickens of the control group I (C) were fed with a grower-type diet based on corn and soybean meal. The chickens from group II received a diet with a 20% seeds of yellow lupine (Lupinus luteus L.), and the group III were fed with the silkworm (Bombyx mori) pupae meal (17%). At 42 days of age, the eight broilers from each group were weighed and slaughtered. The results of broiler growth performance did not show any significant impact of replacement for soybean meal with yellow lupine or silkworm pupae meal and yellow lupine seeds in the grower diets on final body weight and average daily body weight gains. Replacement of 60% of soybean meal with yellow lupine meal (group II) did not have a significant (P>0.05) effect on the analyzed carcass parameters. Introduction of yellow lupine and silkworm pupae to the broiler diets did not have significant impact on the parameters of slaughter yield of carcasses. The content of dry matter in the breast meat of the experimental groups II and III was significantly higher compared to the control group I. There were no significant differences in the content of crude fat in breast muscles, while the value of this parameter in leg muscles was significantly higher in the group III (P<0.01) compared to the control group. There was a significant increase (16%) in the gizzard meat in the group receiving the silkworm pupae meal. Sensory analysis of meat did not show any significant differences in aroma and tenderness. There was a tendency towards greater juiciness and tenderness in the meat of broilers from group III. The obtained results indicate that soybean meal in a grower-type diets can be replaced by silkworm pupae meal and yellow lupine seeds as alternative protein source for growing broilers without negative impact on the performance indices, carcass parameters, chemical composition and sensory quality of the meat.

Keywords: broiler chicken, yellow lupine seeds, silkworm pupae meal, growth performance, sensory quality of meat

Pasture intake and meat antioxidants content of six chicken genotypes organically reared

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The aim of the present research was to evaluate the transfer of antioxidants from pasture to meat of six organically reared chicken genotypes. Chickens of 6 different genetic strains (100 chicks/genotypes: Aviagen Ranger Classic-RC; Aviagen Rowan Ranger-RR; Aviagen Ranger Gold-RG; Hubbard RedJA-RA; Hubbard M22aJAS7-M and Hubbard CJ gen5xJAS7-CY) were organically reared till 81 days of age (spring period). The paddock was constituted of six shelters (0.10 m2/bird) with an external space availability of 4 m2/bird. The grass intake was analyzed by the exclusion pens method at three distance from the shelters (10, 20 and 30 m), and the bioactive compound content (α-β-carotene, lutein and retinol) of pasture, breast and drumstick meat was determined. The explorative behavior of chickens yielded the grass intake of different strains: RR and RA reached the highest distance from the shelter (30 m) and ate a similar quantity of grass for a total of 17.90 and 12.43 g of DM/bird. The RG chickens showed an intermediate trend (13.7, 1.05 and 0.98 g of DM/bird at 10, 20 and 30 m, respectively), whereas the CY and M did not reach 30 m from the shelter. Grass was rich in many antioxidant compounds (mainly α-carotene and lutein): RR and RA chickens showed the highest intake of both lutein (10 and 5-fold, respectively) and α-carotene (10 and 7-fold, respectively), followed by RC, RG, CY and M. The intake of different antioxidants and their respective amount in breast and drumstick showed a non linear trend. The highest α-carotene content was found in the genotypes that showed a lower intake of grass (M and CY), followed by RA, which, instead, showed a higher grass intake. Conversely, for the retinol (a metabolite derivative of the lutein) the RR and RA showed the highest value in breast and drumstick meat (0.37 and 0.28 μg/g, respectively), whereas the RC had the lowest retinol in both cuts (0.07 and 0.13 μg/g). Concluding, the pasture availability is an important factor affecting the quality of organic poultry meat, even if a non linear correlation exists between the intake of bioactive compounds and their content in meat, making to theorize that other factors (oxidative state, bird metabolism, kinetic activity) play a crucial role.

Keywords: chicken genotypes, pasture, antioxidants, oxidative status
Commercial on-farm slaughter could eliminate many potential animal welfare concerns associated with coping, transport, dumping, and shackling of live broilers. However, concerns regarding carcass processing efficiency (defathering and evisceration) and microbiological status following the delay between on-farm slaughter and scalding at the processing plant following transport must be investigated. Market age live broilers obtained from a commercial processing plant were electrically stunned and then bled for 2 min. Carcasses that were delayed prior to scalding were held suspended in shackles for 4 h or 8 h at refrigerated (4°C), room (24-27°C) or body (40°C) temperatures. A control group was processed without a scalding delay. Prior to and following scalding, 6 wing secondary remiges and 6 tail rectrices were extracted and maximum feather retention force (FRF) measured. All carcasses were hard scalded, defathered, and sampled post-evisceration for microbiological analysis. Whole carcass rinses (WCR) were sampled for aerobic plate count (APC), Enterobacteriaceae (EB), Salmonella, and Campylobacter. Ilea and ceca were sampled for Lactobacillaceae, EB, total anaerobes (TA), Salmonella, and Campylobacter. Carcass holding temperature significantly impacted wing and tail FRF for all four treatment groups (P < 0.0001). Carcasses held at 4°C for 4 and 8 h had the highest peak FRF for both wing (3.88 and 3.78 kgf) and tail feathers (1.66 and 1.36 kgf) followed by the non-delayed control group (wing 3.17 and tail 1.44 kgf), the group held at 24-27°C for 4 and 8 h (wing 2.83 and 2.68 kgf, tail 0.96 and 1.04 kgf), and the group held at 40°C for 4 and 8 h (wing 0.84 and 0.63 kgf, tail 0.42 and 0.21 kgf). Following 8 h holding at 24°C significant increases were seen in WCR APC, EB, and Campylobacter (P = 0.0018, 0.0412, 0.0079), and Ileal EB and TA (P = 0.0238 and 0.0103) when compared to control carcasses. However, no significant differences in bacterial counts or prevalence were detected for WCR, Ilea, or cea following a 4 h delay at any of the holding temperatures (P = 0.2494). This study revealed that carcass holding temperature following slaughter for 4 or 8 h significantly impacts the force required for feather removal with higher holding temperatures resulting in decreased FRF and colder holding temperatures increasing FRF. Finally, holding carcasses for 4 h had only minimal impact on carcass microbiology.

Keywords: feather retention force, delayed scalding, Salmonella, Campylobacter, on-farm slaughter

Potential of Pseudomonas putida as biocontrol agent against Salmonella Java in the drinking water system of broiler houses

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Biofilms can provide attachment and protection of other microorganisms (among which pathogens) but can also prevent them from attaching and forming biofilm. This latter situation, wherein living organisms control the presence of other living microbes, is called biocontrol. This potential for both positive and negative interactions among microbes raises the need for in depth characterization of the sociobiology of candidate biocontrol agents (BCAs). Especially the inside of the drinking water system (DWS) is eligible to study interactions since this niche plays an important role in the contamination of broiler chickens and consequently humans with pathogens. More specifically, Pseudomonas putida, which is part of the natural microbiota in the DWS of broiler houses, was evaluated as BCA against the pathogen Salmonella Java, which shows an increasing prevalence in the broiler production chain over recent years. To study the interaction between these species, an in vitro model to simulate biofilm formation on the inside of the DWS of broiler chickens that approached practical conditions was developed and validated. Mono- and dual-species biofilms were grown in 6-well microtiter plates and quantified based on bacterial counts and biomass. Interactions in dual-species biofilms were determined based on cooperation criterion and biodiversity effect. Significant differences in biofilm formation between different strains and biofilm applied inoculum densities were observed, whereby Salmonella Java was evaluated as the best biofilm former among the tested strains and biofilm formation increased with increasing inoculum density. Biofilm formation by Pseudomonas putida and Salmonella Java was always characterized by competitive interaction, independent of the Pseudomonas putida strain, Salmonella Java inoculum density and application order. This study provided the first results indicating the potential of Pseudomonas putida as a BCA against Salmonella Java in the broiler environment. These results could be used in further research concerning alternative methods to eliminate pathogens in primary animal production environments and to prevent the animals and humans from being infected.

Keywords: biofilms drinking water systems, broilers, Salmonella Java, Pseudomonas putida, biocontrol agent

Repeated disinfectant use in broiler houses does not affect disinfectant and antibiotic susceptibility in Escherichia coli field isolates

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Disinfectants are frequently used in animal production to reduce or eliminate the load of bacteria and viruses in buildings and equipment associated with the housing or transportation of animals. There are growing concerns that the use of disinfectants would select for resistance to antibiotics and disinfectants. Therefore, the aim of this study was to determine the effect of repeated use of different disinfectants on the disinfectant and antibiotic susceptibility of bacteria under practical conditions. Escherichia coli (E. coli) was isolated from environmental samples taken after cleaning and disinfection at an experimental broiler farm over a one-year period. The E. coli isolates were screened for their susceptibility to 14 antibiotics and four disinfectants by determining the minimal inhibitory concentration (MIC). Results showed a high resistance level for amoxicillin (80%), enrofloxacin (70%) and trimethoprim (61%). A high and moderate antibiotic resistance was found for tetracycline (28%), ciprofloxacin (19%) and nalidixic acid (16%). Disinfectant susceptibility did not change over time and was not dependent on the used disinfection product. Compared to in-use concentrations of formaldehyde, benzalkoniumchloride (QAC) and a peracetic acid and hydrogen peroxide formulation, all E. coli strains were susceptible indicating that the proper use of disinfectants would not select for disinfectant resistance. Moreover, no association could be found between the use of disinfectants and antibiotic resistance. In conclusion, the findings of this study suggest that repeated and proper use of disinfectants in broiler environments does not select for antibiotic resistance nor reduce disinfectant susceptibility.

Keywords: disinfectants, antibiotic resistance, disinfectant susceptibility, Escherichia coli, broiler production

Development of a functional chicken meat-based snack by 3D food printing: effects of starch addition and cooking methods

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Three dimensional (3D) food printing is an emerging computer-aided additive manufacturing approach used for developing newly designed food products with specific and challenging characteristics not attainable by conventional processing technologies. The aim of this study was to develop and evaluate in terms of quality, a new healthy chicken meat-based snack with an appealing shape, specific structure and taste using 3D printing and to analyze the effects of starch addition to a basic formulation that was processed by two cooking methods, rapid air cooking (RAC) or oven cooking (OC). A 3D printer with extrusion printing technology was used for this purpose. Two formulations were prepared using chicken meats, powders of cumin, garlic and turmeric, milk, and butter with and without wheat starch addition where the latter was used as control. Based on previous studies, 2.5% (w/w) starch level was used to obtain the best results in terms of printability characteristics and product quality. The 3D printed flower-shaped chicken meat products were cooked at 160°C for 3 min with the RAC whereas the OC was used at 160°C for 8 min. The pH values for RAC lightness-L*, redness-a* and yellowness-b*, texture profile analysis, and consumer acceptability test were conducted. No effect of cooking method and starch on the pH value (p > 0.05) was observed. RAC resulted in lower (p < 0.05) L* value, while starch had no significant effect on L* value (p > 0.05). Starch addition led to lower a* and b* values than the control (p < 0.05). Hardness and chewiness values were higher in the starch added group using RAC (p < 0.05). In sensory analysis, there was no significant effect of starch addition and cooking method on appearance, color, texture, and general acceptability, whereas the samples exposed to RAC received higher flavor scores (p < 0.05). In conclusion, starch addition to improve the printability formulation did not have a negative impact on the product characteristics. Due to shorter processing time, RAC is the recommended one because, in addition to delivering a high quality product, it is energy and time efficient. The present study indicates that 3D printing enables to develop an innovative snack food with a sophisticated and appealing shape, enhanced nutritional value and acceptable sensory characteristics. 3D food printing offers an excellent alternative to process chicken products that could drive the poultry meat industry to become more competitive in today’s global food market.

Keywords: 3D food printing, Healthy food design, Functional chicken meat product
Consumer preferences for chicken meat produced using micro-algae or insect meal

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Due to changing diets, a global protein shortage is predicted by 2050; therefore, research has invested in identifying new protein sources, especially in animal feed. Two such protein sources are spirulina and insect meal. This study focused on determining consumer preferences for raw poultry meat (chicken breast) produced with two novel protein sources using a discrete choice model. Photos of the novel-produced chicken breasts and of a standard soybean-fed product were modified to depict a typical package of chicken breast available at a German grocery store. Chicken breast produced with spirulina is an orange color whereas black soldier larvae (insect meal) results in a slightly more yellow color compared to the standard product. The assessment took place via an online survey distributed by a commercial panel provider and respondents were recruited based on age (18-75 years), sex (50/50 male/female) and being chicken breast purchasers. In a split-sample, 1074 German consumers completed the choice task either blind (n=540) or after receiving information on the protein feed used in production and identification as “fed with…” (n=534). Results were computed using a mixed-logit model, where price was a fixed variable and all others were random. Uninformed consumers rejected chicken breast produced with spirulina when faced with the choice between the new or the standard product; they had a reduced average willingness-to-pay of 1.04€, comparatively. Chicken breast produced with insect meal was preferred compared to the standard product in a blind choice scenario. The preference increased substantially when consumers received information about insects in poultry diets and the product was identified as “fed with insects.” The average willingness-to-pay compared to the standard the three BMM, with higher incidence among females than males. On day 39, the 2 farms differ significantly in mean BW (2060g vs. 2240g), but the farms hardly differed in the incidence of the 3 BMM. The incidence of WB (mild-severe) was higher among males (45%) than females (18%), whereas the incidence of mild-severe SB was higher among females (63%) than males (34%). Within each sex, the birds with WB had significantly lower pH at 15 minutes and 24 h postmortem than the birds with SB. The occurrence of SB and WB was not associated with body and breast weights. These results suggest the need to further investigate the effect of sex and weight on each BMM.

Keywords: broiler, breast muscle myopathies, wooden breast, paghetti breast


textual content extracted
**Effect of chickens breed, lysine depletion and feed form on breast meat quality**

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Meat quality is an important issue with fast-growing broilers. Severe white striping (WS) and wooden breast (WB) cases are getting more frequently reported in broiler production. To understand the causes of these defects, a study was conducted to evaluate 42 birds. In the study, birds were divided into 8 groups, each receiving pellets with standard nutritional levels (RP) and those supplemented with either probiotics (Bacillus subtilis) or proteases (papain/bromelain), and supplemented with different protein sources (native soybean meal - NORM, defatted Hermetia illucens - HI). The diets were fed ad libitum to Ross 308 broilers at 250 g/kg. The diets were supplemented with either probiotics or enzymes, or both.

The results showed that the combination of probiotics and enzymes had the most significant impact on reducing meat defects. The NORM diet resulted in an 8% higher WB and WS defects compared to the HI diet, with no severe cases. RCM and RPL groups had less WB and WS defects: -53% of WB and WS compared to RP, with no severe cases. However, the data suggested a decreasing trend (P < 0.01) for WB fillets of the E group (20.1 ± 1.9 N) compared to BW fillets belonging to the B group (27.7 ± 2.1 N). Fillets marinated with the proteases exhibited a mushy, unappealing appearance once cooked. From this study, it emerged that the use of marinated solutions containing proteolytic enzymes was an effective strategy to reduce texture issues in BW intact muscle products.

**Keywords:** Wooden Breast, texture, tenderizing strategies, exogenous proteases

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**Feeding crude corn oil as by-product of ethanol industry to broiler chicken**

**Márta Erdélyi**, Róbert Kecskés, Zsolt Ancsin, Csaba Ferney, Andrea Bócasai, Krisztian Balogh, Miklós Mizés

Department of Animal Nutrition, Institute of Basic Animal Sciences, Szent István University, Gödöllő, Hungary

As corn based ethanol production is a dynamically increasing business, its by-products have good potential as feedstuffs for livestock production. In 2017, two tons of corn was processed only in Hungary, which resulted in 20,000 tonnes of crude corn oil production. It is used mainly in monogastric nutrition as an energy supplement, but its effects on product quality is not widely investigated so far. In our study 120 newly hatched cockerels were randomly divided in four groups. Control birds were fed ad libitum intensive broiler grower diet. While in the three experimental groups - namely FSFO, FCFO, HTCCO - on week 3-4, 4% on week 5-6-6% of fresh sunflower oil, fresh crude corn oil or heat treated crude corn oil was included in the control diet, respectively. Production traits - live weight, daily weight gain, feed intake – and certain product quality traits - meat, grease, skin colour, water holding capacity, pH, tenderness – were measured. Also, redox status in the liver was analyzed with parameters of malondialdehyde concentration, reduced glutathione concentration and glutathione peroxidase activity.

**Keywords:** corn oil, broiler chicken, meat quality, glutathione redox system

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**Potential of probiotic (Bacillus subtilis) and carbohydrase enzymes in improving nutritional value of pea meal in broiler diets**

**Paweł Konieczka**1, Jan Jankowski1, Krzysztof Kozlowski1, Katarzyna Zabek2, Marcin Barszcz2, Stefania Smulińska2

1Department of Poultry Science, University of Warmia and Mazury in Olsztyn, 10-719, Olsztyn, Poland
2Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland

Peas are a valuable source of dietary protein and energy for poultry. The carbohydrate fraction (non-starch polysaccharides) of peas is poorly digested in chickens and may serve as substrate for bacterial fermentation in the lower part of the gastrointestinal tract. The aim of the study was to investigate if partial replacement of soybean meal by pea meal in broiler diet is possible without affecting shift in abundance of pathogenic bacteria in caecum. Pea meal (cv. Model, grown in Poland) was included in a wheat-soybean meal based diet at 250 g/kg. The diets were un-supplemented (control) or supplemented with either probiotic (Bacillus subtilis), carbohydrase enzymes (200 U/kg xylanase and 10 U/kg β-glucanase in feed) or both. The diets were fed ad libitum to Ross 308 broilers aged 9–28 days, kept in the individual cages. Birds were sacrificed after two additional days, gastrointestinal tracts were then excised and caecum digesta was analyzed using 16S rRNA gene amplification for the Escherichia coli, Salmonella spp., and Campylobacter jejuni relative (to the total bacteria) abundance. Feeding the pea-based diet supplemented with the probiotic combination fed utilization, due to higher feed intake, but addition of enzymes to pea containing diet partially ameliorated this effect. The abundance of Escherichia coli and Campylobacter jejuni in the caecum digesta was not affected by the dietary treatments (P > 0.05) while, the abundance of Salmonella spp. in all experimental groups was significantly lower than that of the control group.

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**Feeding crude corn oil as by-product of ethanol industry to broiler chicken**

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**Keywords:** corn oil, broiler chicken, meat quality, glutathione redox system
Keywords: Broiler chicken, probiotic, pea meal, gut microbiota, enzymes

Positive Effect of 25-OH-D3 on broiler meat quality defects

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In the last decade, muscle abnormalities have appeared in the broiler industry (white striping - WS, Wooden Breast - WB and Spaghetti Meat- SM) mainly affecting heavy broiler production. However, the origin of these problems remains unclear. Vitamin D3 plays a crucial role in muscle development (Ceglia et al. 2009). A more bio-available source of vitamin D3, 25-OH-D3, has been demonstrated to improve the production of vitamin D receptors (VDR) and myogenic regulator factors (Ceglia et al. 2015) leading to improved breast meat yield and protein content of pectoralis major (Michalczuk et al. 2010). This study aimed to investigate whether increased 25-OH-D3 (Rovimix® Hy:DB) dietary supplementation reduced meat quality defects. For this purpose, 42 flocks (14000 male / flock) from nine farms of heavy Ross 308 broilers received two different diets. Each group received the equivalent amount of vitamin D3 (Rovimix® Hy:DB) of 115, 75 and 62.5 µg/kg for starter, grower 1, grower 2 and finisher respectively. The control group (CG) received their regular diet already supplemented with 25-OH-D3 during starter and grower phases (25 µg and 40 µg/kg feed respectively). The "25-OH-D3" group received a higher amount of 25-OH-D3 during the whole feeding period (115, 107.5, 67.5 and 56.5 µg/kg of feed for each feeding phase respectively). Meat defects in 100 pectoralis major per flock were assessed and classified at slaughter into three classes for WS (Normal, Medium, Severe) and two classes for SM (with or without) following the slaughter plant usual scoring scale. No statistical difference was observed for zootechnical performance, but the 25-OH-D3 group showed improved DWG (+0.40g/d) and flock homogeneity (11.5% vs 12.1% for the control group). At slaughter, the down grade rate was lower for the 25-OH-D3 group (P<0.06) and drumstick meat yield was significantly higher (34.2% vs 33.5%, P<0.03). Finally, WS was significantly lower for the 25-OH-D3 group (P<0.001): 63.6% pectoralis major were normal vs 49.8% for the control group. The lack of marked differences in WS and WB incidence here could be explained by the regular use of 25-OH-D3 in the control diet, which is a common practice on these farms.

Keywords: Meat quality defects, 25-OH-D3, vitamin D3

Effect of zinc source and level on broiler carcass defects at market age

Cibile Torres, Francisco Fernandez, Tim Horne, Marco Rebollo

Zinpro Corporation

Zinc (Zn) is an essential trace mineral for animals and is involved in several biochemical processes that support skin and bone health. A total of 1296 day-old male Ross 308 chicks were placed to investigate the effect of Zn dietary supplementation on carcass quality of broilers to market age. The study was a complete block design with 12 pen replicates per treatment, where broilers were randomly distributed across 48 pens and received one of four dietary treatments during starter (1-10 d, mash), grower (10-21 d, mash) and finisher periods (21-34 d, pellet). Experimental diets, based on wheat and soybean meal were formulated with supplemented Zn levels of 40 ppm as ZnSO4 (40-ZnSO4); 40 ppm as Zn amino acid complex (40-ZnAAC); 80 ppm as ZnSO4 (80-ZnSO4); and 80 ppm combination of 40 ppm ZnSO4 + 40 ppm Zn-AAC (80-ZnSO4+ZnAAC). Birds had ad libitum access to feed and water. At 35 days, 120 broilers per treatment were randomly selected for carcass measurements. Carcass damages were assessed before the chilling process. Measurements included: a) bone protrusion (1: pop-out mild with closed skin; 2: pop-out and open skin; 3: complete bone fracture), b) skin integrity (1: superficial scratches; 2: <4 cm skin rupture; 3: extensive skin rupture) c) hematoma (mild: <2cm bleeding; severe: ≥2cm and >6cm bleeding). The incidence of bone protrusion score 3 was lowest with 80-ZnSO4+ZnAAC and highest at 40-ZnSO4 (9.2% vs 1.8% respectively, P<0.05); birds from 40-ZnAAC and 80-ZnSO4 showed intermediate and not significantly different values (3.3 vs 4.2%, respectively, P>0.05). Birds fed Zn from Zn-ACC had higher incidence of bone protrusion score 1. Higher percentage of carcasses with intact skin were seen when feeding 80-ZnSO4+ZnAAC compared to 40 ppm as Zn-AA or ZnSO4. The incidence of superficial scratches in skin was highest at low Zn level, regardless of Zn source, and lowest at 80-ZnSO4+ZnAAC. Broilers fed 80-ZnSO4 tended to have more carcasses with superficial scratches compared to 80-ZnSO4+ZnAAC (P>0.07). The incidence of mild and severe hematomas in carcasses were reduced when diets were supplemented with 40-ZnAA alone or in combination with ZnSO4. Replacing Zn from sulfate with Zn-AAC can help decrease defects in broiler carcasses.

Keywords: Zinc, performance mineral, carcass quality
Introduction

Recently, increasing awareness of consumers on healthy diet drives meat industry to utilization vegetable oils instead of animal fat (Alejandre et al. 2019a). Using vegetable or marine based liquid oils in product formulation without any structural modification, caused undesirable changes in meat products, while increasing unsaturated fatty acid composition (Ilikkan et al. 2009; Dominguez et al. 2017). Thus solidifying liquid oils with different methods such as emulsification could become an important way to avoid the negative effects of animal fat replacement (Salcedo-Sandoval et al. 2015, Herrero et al. 2017) 9 days, however, since liquid oils are considered highly perishable to oxidative changes, emulsification techniques might be insufficient to protect against oxidation. In such circumstances, incorporating one or two antioxidants into gel emulsion formulations exhibit better oxidative stability than no antioxidant added samples (Alejandre et al. 2019b). Rosemary and ascorbic acid are antioxidants that have different inhibition mechanisms and combined use of these antioxidants have been reported to have promising effects on oxidative changes (Hwang et al. 2017). In this study, our purpose was to discuss the effects of using two different antioxidants in gelled emulsion formulation model system chicken emulsions.

Material and methods

Hot-set emulsion gels were prepared according to Peyato et al. (2014) with some modifications by using, egg white powder, inulin, gelatin and peanut and fluxseed oil mixture. Four different model system meat emulsions (MSME) were formulated, and prepared following the procedure reported by Collados et al. (2008) with modifications; Control (C) containing 9.75% beef fat and no antioxidant, other samples which were added gelled emulsions are; no added antioxidants, rosemary oil in oil phase, ascorbic acid in water phase and, rosemary+ ascorbic acid in both phase are termed as GE, R, A and R/A respectively. Chicken samples were stored at 4°C and analyzed on 0, 3rd and 7th days. Total moisture (AOAC, 2012), ash (AOAC, 2012) and fat (Flynn, other samples which were added gelled emulsions are; no added antioxidants R/A samples had the highest initial a* values (P<0.05). b* values of all samples descended at the end of the storage but, R and R/A samples had the highest b* values. Depending of this results it can be said that meat products color could be preserved with gelled emulsion and oxidative changes.

Results

Chemical compositions and pH values of CE were given in Table 1. Moisture, protein, fat and ash contents of samples were changed between 69.46–71.71%, 12.99–14.58%, 12.36–12.76% and 2.94–3.14% respectively. Differences in moisture, protein and fat contents were dependent to emulsion formulation on the model system chicken emulsions. Protein content of the C samples formulated with beef fat (P<0.05). These results could be associated with the emulsion mechanisms and combined use of these antioxidants have been reported to have promising effects on oxidative changes (Hwang et al. 2017). In this study, our purpose was to discuss the effects of using two different antioxidants in gelled emulsion formulation model system chicken emulsions.

Table 1. Chemical composition, pH and color values of model system chicken meat emulsions

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>pH-0th day</th>
<th>pH-3rd day</th>
<th>pH-7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>71.71±</td>
<td>12.99±0.64*</td>
<td>12.36±0.05</td>
<td>2.94±0.01</td>
<td>6.16±0.01</td>
<td>6.22±0.01</td>
</tr>
<tr>
<td>GE</td>
<td>72.14±</td>
<td>14.19±0.09*</td>
<td>12.98±0.42</td>
<td>3.08±0.01</td>
<td>6.15±0.01</td>
<td>6.23±0.01</td>
</tr>
<tr>
<td>R</td>
<td>71.24±</td>
<td>14.13±0.32*</td>
<td>12.67±0.04</td>
<td>3.06±0.01</td>
<td>6.11±0.01</td>
<td>6.20±0.01</td>
</tr>
<tr>
<td>A</td>
<td>71.85±</td>
<td>14.48±0.29*</td>
<td>12.57±0.47</td>
<td>3.04±0.01</td>
<td>6.12±0.01</td>
<td>6.22±0.01</td>
</tr>
<tr>
<td>R/A</td>
<td>69.88±</td>
<td>14.22±0.41*</td>
<td>12.76±0.32</td>
<td>3.14±0.01</td>
<td>6.15±0.01</td>
<td>6.22±0.01</td>
</tr>
</tbody>
</table>

Peroxide and TBARS values of model system chicken meats

<table>
<thead>
<tr>
<th>Peroxide</th>
<th>pH-0th day</th>
<th>pH-3rd day</th>
<th>pH-7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.95±0.10*</td>
<td>5.96±0.55*</td>
<td>7.98±0.11*</td>
</tr>
<tr>
<td>GE</td>
<td>6.96±0.90*</td>
<td>4.98±1.00*</td>
<td>7.96±0.22*</td>
</tr>
<tr>
<td>R</td>
<td>6.91±1.01*</td>
<td>4.44±0.51*</td>
<td>5.63±0.59*</td>
</tr>
<tr>
<td>A</td>
<td>5.91±0.05*</td>
<td>4.97±0.98*</td>
<td>5.99±0.22*</td>
</tr>
<tr>
<td>R/A</td>
<td>3.39±0.48*</td>
<td>4.61±1.17*</td>
<td>6.00±0.04*</td>
</tr>
</tbody>
</table>

TBARS

<table>
<thead>
<tr>
<th>TBARS</th>
<th>pH-0th day</th>
<th>pH-3rd day</th>
<th>pH-7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.25±0.01*</td>
<td>0.25±0.03*</td>
<td>0.94±0.03*</td>
</tr>
<tr>
<td>GE</td>
<td>0.71±0.03*</td>
<td>0.71±0.02*</td>
<td>0.83±0.03*</td>
</tr>
<tr>
<td>R</td>
<td>0.62±0.02*</td>
<td>0.97±0.00*</td>
<td>0.86±0.03*</td>
</tr>
<tr>
<td>A</td>
<td>0.57±0.01*</td>
<td>0.72±0.08*</td>
<td>0.48±0.22*</td>
</tr>
<tr>
<td>R/A</td>
<td>0.87±0.01*</td>
<td>1.04±0.33*</td>
<td>0.53±0.01*</td>
</tr>
</tbody>
</table>

Data are presented as the mean values of replications ± standard deviation. abc: Means with the different letter in the same column are significantly different (P<0.05). Data are presented as the mean values of replications ± standard deviation. xyz: Means with the different letter in the same column are significantly different (P<0.05).

References


Effects of using egg shell powder as phosphate replacer on quality properties of chicken patties

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Abstract

Phosphates are used in meat products to enhance water holding capacity and protein solubility by increasing pH. However negative effects of phosphates on human health drive meat industry searching natural phosphate replacers. The aim of this research was to investigate the effects of using egg shell powder (ESP) or mixture of egg shell powder (ESP)and egg white powder (EWP) as phosphate replacers on some quality characteristics of chicken patties. Chicken patty samples were subjected to three treatments, as follows: control (0.5% phosphate added), 0.5% egg shell powder added (ES) and 0.5% egg shell powder +0.25% egg white powder added (ESW). Chemical composition and technological properties (cooking yield, water holding capacity, fat and moisture retention) were evaluated in comparison to control patties. Fat binding capacity of ESW samples was better than EWP. Fat and ash values of uncooked and cooked samples are presented in Table 2. In uncooked samples total chemical composition of the raw and cooked patties were determined according to AOAC (2012). Protein content value of egg shell powder resulted similar protein content with the control samples (P<0.05). pH of egg shell powder addition resulted higher protein solubility because of the high pH. The results showed that the combination of ESP with EWP could be an alternative natural additive for phosphate-free meat products.

Keywords: egg shell powder, phosphate replacer, phosphate-free, chicken patty

Introduction

Phosphates are a chemical synthetic analogue. Phosphates used in meat products are mainly to improve the product yield by enhancing the water binding property, quality properties such as texture, color, flavor parameters and antioxidant functions. Other beneficial effects of phosphates are; stabilization of emulsions and the texture of meat products by increasing the extraction of salt-soluble proteins based on increasing ionic strength and charges, and reduction of lipid oxidation via their metal chelating activity, which subsequently inhibits off-flavor development (Sebranek, 2009). Despite these multifunctional benefits, phosphates has decreased in the last decade because of poor consumer perception associated with the health risks (Kim et al., 2017; Petracchi et al., 2013; Wuhanabe et al., 2016). Therefore, meat industry has taken steps to find suitable alternatives to meet the consumer demands (Öztürk and Serdaroglu, 2017). Many previous studies have attempted to improve the functionalities of meat products by using various functional ingredients, such as dried plums (Jarvis et al., 2012; Jarvis et al., 2015), functional carbohydrates, including guar gum, carrageenan, alginic acid and chitosan (Park et al., 2008), 0.2% oyster shell calcium powder, 0.3% egg shell calcium powder, and 0.25% whey protein concentrate (Jeong, 2018). In this study, eggshell powder and egg white powder were used as natural phosphate substitution. To the best of our knowledge, yet no research has been performed regarding utilization of egg shell powder and/or egg white powder as phosphate replacer in chicken patties. Therefore, the aim of this study was to evaluate the effect of using egg shell powder as phosphate substitution on some quality properties of chicken patties.

Material and Methods

Three different chicken patties were formulated; In control sample, 0.5 g/100 g food grade sodium tripolyphosphate (control) was added. Other treatments were formulated with: 0.5% egg shell powder (ES), 0.5%+1/3% egg white powder (EWP) as phosphate replacers. Salt (1.5%) and ice (15%) was added to all formulations. Chicken skin was used (15%) as fat source. Chicken mixed (breast and thigh) and skin were grounded through a 3 mm plate grinder. 700 grams of batches of appropriate amounts of mixed (breast and thigh) and skin were grounded through a 3 mm plate grinder. 700 grams of batches of appropriate amounts of the samples was determined using a universal nitrogen analyzer (FP 528 LECO,USA) based on the Dumas method. Fat content was evaluated according to Flynn and Bramblet (1975). Percent cooking yield was determined by calculating weight differences for samples before and after cooking (Murphy et al., 1975). Fat retention was calculated according to Murphy et al. (1975). The moisture retention was determined according El-Magoli et al. (1996). Water holding capacity was determined according to Hughes et al., (1997) with slight modifications. The Bradford method was used to measure protein solubility.

Results

Chemical composition and pH values of uncooked and cooked patties are presented in Table 2. In uncooked samples total moisture, protein, fat and ash contents changed between 72.28-75.66%, 13.84-16.03%, 8.14-9.43% and 2.71-3.14%, respectively. The differences of formulation resulted significant changes in moisture, protein and ash content of raw samples (P<0.05) while no effect was recorded in fat content (P>0.05). ESW treatment had higher moisture content compared to others (P<0.05). The combined use of egg shell powder (ESP) and egg white powder (EWP) resulted similar protein content with the control samples (P>0.05). pH value of uncooked samples was found between 6.17 and 6.21. In cooked samples total moisture, protein, fat contents ranged between 72.20-75.66%, 15.64-18.79% and 8.36-10.22% respectively. No significant differences were found in pH values of cooked samples, pH values ranged between 6.17 to 6.29.
ESW treatment which egg shell powder and egg white powder is used as phosphate replacer.

Table 3 Cooking characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.0±0.15</td>
<td>15.39±0.55</td>
<td>9.43±0.37</td>
<td>3.14±0.31</td>
<td>6.20±0.01</td>
</tr>
<tr>
<td>ES</td>
<td>73.58±0.56</td>
<td>13.84±0.35</td>
<td>8.14±0.64</td>
<td>2.71±0.09</td>
<td>6.17±0.00</td>
</tr>
<tr>
<td>ESW</td>
<td>75.66±0.43</td>
<td>16.03±0.53</td>
<td>8.89±1.62</td>
<td>2.89±0.10</td>
<td>6.16±0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.59±1.87</td>
<td>18.79±1.27</td>
<td>9.99±0.73</td>
<td>3.75±0.16</td>
<td>6.23b</td>
</tr>
<tr>
<td>ES</td>
<td>70.72±0.61</td>
<td>16.38±1.38</td>
<td>8.36±1.07</td>
<td>3.13±0.10</td>
<td>6.17c</td>
</tr>
<tr>
<td>ESW</td>
<td>72.13±1.63</td>
<td>15.64±0.57</td>
<td>10.22±3.98</td>
<td>3.04±0.11</td>
<td>6.29a</td>
</tr>
</tbody>
</table>

Control samples formulated with STTP had lowest moisture retention (60.77%) and fat retention (95.01%) values. WHC was recorded 75.49, 71.53 and 81.06% for control, ES and ESW treatments respectively. Protein solubility significantly affected by the formulation, the highest protein solubility (1096.29 µg protein/ml) was recorded in ESW treatment which egg shell powder and egg white powder is used as phosphate replacer.

**Discussion**

This study was conducted to investigate egg shell powder and combination of egg shell powder and egg white powder as phosphate replacements in chicken patty formulation. Cooking process increased pH protein, fat and ash contents of samples while decreased moisture content due to cooking loss. Addition of ESP and EWP increased the pH value in cooked samples and the highest pH value was observed in ESW group (P<0.05). The pH of EWP was above the isoelectric pH (approximately 5.0) of myofibrillar proteins. At the isoelectric pH, proteins have a net zero charge, attractive forces predominate, and molecules tend to associate, thus resulting in insolubility. Above the isoelectric pH, the net charge is negative and solubility is enhanced in ESP treatment. Alkalai treatment usually increases the solubility of the meat proteins by allowing the proteins to dissociate (Zayas, 1997). Cooking characteristics improved by the addition of ESP and EWP. With the addition of ESP fat retention was enhanced, but the highest fat retention was seen in ESW samples where the combination of egg shell and egg white powders is used. Retaining fat within the matrix of meat products during processing is necessary to ensure sensory quality and acceptability (Serdaroglu et al., 2017). The lowest fat retention was found in control sample since it has higher fat content and due to melting of fat globules higher leakage was observed during cooking. Overall, the combination use of egg shell and egg white powders rather than the addition of egg shell powder alone improved cooking parameters and protein solubility when compared with the control treatment containing synthetic phosphate.

**References**


**Table 3** Cooking characteristics and protein solubility of patties

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cooking Yield (%)</th>
<th>WHC (%)</th>
<th>Moisture Retention (%)</th>
<th>Fat Retention (%)</th>
<th>Protein Solubility (µg protein/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.91±6.31b</td>
<td>75.49b</td>
<td>60.77b</td>
<td>95.01b</td>
<td>912.12b</td>
</tr>
<tr>
<td>ES</td>
<td>91.58±0.46</td>
<td>71.53b</td>
<td>65.27b</td>
<td>95.56b</td>
<td>849.73b</td>
</tr>
<tr>
<td>ESW</td>
<td>92.30±1.07</td>
<td>81.06b</td>
<td>66.05b</td>
<td>97.50b</td>
<td>1096.29b</td>
</tr>
</tbody>
</table>

Cooking yield varied between 89.91 – 92.30%, the lowest cooking yield was recorded in control samples, this finding has been verified by the moisture and fat retention values, control samples formulated with STTP had lowest moisture retention (60.77%) and fat retention (95.01%) values. WHC was recorded 75.49, 71.53 and 81.06% for control, ES and ESW treatments respectively. Protein solubility significantly affected by the formulation, the highest protein solubility (1096.29 µg protein/ml) was recorded in ESW treatment which egg shell powder and egg white powder is used as phosphate replacer.

**Discussion**

This study was conducted to investigate egg shell powder and combination of egg shell powder and egg white powder as phosphate replacements in chicken patty formulation. Cooking process increased pH protein, fat and ash contents of samples while decreased moisture content due to cooking loss. Addition of ESP and EWP increased the pH value in cooked samples and the highest pH value was observed in ESW group (P<0.05). The pH of EWP was above the isoelectric pH (approximately 5.0) of myofibrillar proteins. At the isoelectric pH, proteins have a net zero charge, attractive forces predominate, and molecules tend to associate, thus resulting in insolubility. Above the isoelectric pH, the net charge is negative and solubility is enhanced in ESP treatment. Alkalai treatment usually increases the solubility of the meat proteins by allowing the proteins to dissociate (Zayas, 1997). Cooking characteristics improved by the addition of ESP and EWP. With the addition of ESP fat retention was enhanced, but the highest fat retention was seen in ESW samples where the combination of egg shell and egg white powders is used. Retaining fat within the matrix of meat products during processing is necessary to ensure sensory quality and acceptability (Serdaroglu et al., 2017). The lowest fat retention was found in control sample since it has higher fat content and due to melting of fat globules higher leakage was observed during cooking. Overall, the combination use of egg shell and egg white powders rather than the addition of egg shell powder alone improved cooking parameters and protein solubility when compared with the control treatment containing synthetic phosphate.

**References**


A novel way to suppress growth of *Campylobacter* in chickens prior to processing

Tamsyn M Crowley, Ben Wade, Sarah Shigdar, Anthony Keyburn

Food poisoning is quite common in Australia affecting an estimated 4.1 million cases each year (NSW Food Authority). Of the major causes of food poisoning *Campylobacter* and *Salmonella* are responsible for most of the bacterial food poisoning outbreaks in Australia. Poultry is known to be one of the major sources of *Campylobacter* and *Salmonella*, these bacteria occur naturally in chickens and generally do not affect their health. In order to reduce the risk of bacterial food borne illness there is a necessity to reduce the commensal levels in meat chickens prior to processing. Evidently, the lower the contamination of these bacteria at the beginning of the process line, the easier it will be to minimise the bacterial levels at the end of the processing line. Our laboratory has employed a number of techniques to provide a cheap, specific and reliable way of reducing the *Campylobacter* load in broilers prior to processing, ensuring an overall reduction/elimination of *Campylobacter* contamination and ultimately a decline in human illness caused. We have employed a multiplex approach that will enable multiple strains of *Campylobacter* to be targeted and we envisage that our resulting treatment will be able to be delivered to chickens in drinking water. In addition, the same technology can be used to generate strain specific tests to detect *Campylobacter* in poultry. This approach has the potential to be used to reduce the levels of other food poisoning bacteria such as *Salmonella*, *E. coli* and *Listeria*.

**Keywords:** *Campylobacter*, human health, broiler

**XXIV European Symposium on the Quality of Poultry Meat**

**5 Minutes Oral Presentations**
Using a probiotic to ensure food safety

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Due to growing awareness food safety has improved greatly over the years. However, contamination with bacteria such as Salmonella is a constant risk and a concern to producers and consumers alike. Probiotics are an interesting option to control these zoonotic bacteria in the production process, via direct (bio-active substances) and indirect (competitive exclusion) mechanisms. As a result the risk of final product contamination is reduced. Bacillus licheniformis (DSM 28710, B-Act®), is a good example of such a probiotic. The facility of Southern Poultry Research (USA) was used to divide 800 Male Cobb broilers at random over two treatments: a control group without probiotic supplementation and a B-Act® group receiving 0.5 kg B-Act®/mton of feed, or 1.6 x 10^9 CFU Bacillus licheniformis/gram of feed. The birds were housed in 16 pens of 50 birds, resulting in 8 replicates per treatment. On the first day of the trial 25 broilers per pen (50% of the birds) were orally inoculated with 2 cc nalidixic acid resistant to Salmonella Heidelberg. Trial duration was 42 days, during which weight and feed intake was recorded to allow for feed conversion ratio (FCR) calculation. To determine the presence of Salmonella in the environment swab samples were collected from all pens on days 14 and 42. Finally ten birds per pen were randomly selected at the end of the trial, euthanized and the ceca aseptically removed for Salmonella isolation.

Total weight gain and feed conversion ratio were significantly (P<0.05) improved in the B-Act® group, with a difference compared to the control of respectively 67 grams (2117 g vs. 2050 g) and 0.072 (1.906 vs. 1.978). All environmental swab samples were positive for Salmonella indicating a strong Salmonella Heidelberg challenge. However, there was a significant (P<0.05) reduction in Salmonella prevalence when B-Act® was supplemented, with percentage differences of 26% for inoculated birds (62% vs. 36%) and 18% for contact birds (56% vs. 38%). The addition of B-Act® to the diet at a rate of 0.5 kg/mton did not only significantly (P<0.05) improve growth performance and feed conversion ratio, it also had significant effects on the reduction of Salmonella prevalence in broiler chickens. As such B-Act® can contribute to food safety and the reduction of pathogenic bacteria in poultry, whilst at the same time supporting optimal production.

Keywords: Bacillus licheniformis, B-Act®, Salmonella, Food safety, Probiotics
The comparison of production and meat quality of turkey crossbreds from Old Hungarian and commercial breeds

Rubiha Tündé Szabó1, Árpád Drobnyák1, Mónika Heininger1, Ágnes Zimborán1, László Bődi1, Károly Kustos1, Ádám Csányi1, Mária Kovács Weber1

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Nowadays, strategies of breeders cost-efficiency and higher production values in the shortest possible time. To meet goals, meat type hybrids with high breast meat yields were developed in turkey breeding. There is a growing demand for better quality and more traditional products. In the case of turkey breeds, this is represented by the Old Hungarian Bronze and Copper varieties. However, these varieties are not compatible with the contemporary production challenges, therefore there is a need for crossbreds. The aim of this study was to investigate the meat production and quality of crossbreds from old Hungarian turkey breeds. Four crossing genotypes were established: Converter hybrid sire x Bronze dam, Converter hybrid sire x Copper dam, Bronze sire x Converter hybrid dam and Copper sire x Converter hybrid dam. 2600 turkey pipes were placed, separated by sex and crossed breed construction. All birds received wing tags and were raised under similar conditions on deep litter (1.5-2.5 cm wood shavings). The feed content varied with age and included breeder-type feed. The live weight was measured from week 18 to 24 weekly and at the slaughterhouse. The birds were slaughtered at the age of 28 weeks and the weight of the individual pieces of carcass (breast, leg) was measured. The following meat quality parameters were investigated in breast: colour, drip loss, pH, electrical conductivity, kitchen technology losses, shear force value. At week 24, Converter hybrid x Bronze had the highest live weight (14.22 kg), Converter hybrid x Copper and Bronze x Converter groups reached the same weight (12.99 kg, 12.14 kg), the lowest live weight was measured in the Copper x Converter group (10.02 kg). The Converter hybrid x Copper had the lightest (L=52.49), but the Copper x Converter genotype had the reddest (a*=4.32) breast meat. The favourable drip loss was in the Converter x Copper (6%) and Converter x Bronze (6.7%) groups. As a conclusion, constructions with the paternal line produced the highest results in both production and meat quality. From these, Converter hybrid sire x Bronze dam genotype had more favourable meat production compared to the Converter hybrid sire x Copper dam genotype. On this basis, if the breeding goal is directed to a larger carcass, it is worthwhile to use a meat hybrid paternal line for better results. Further breeding and testing are recommended because of excellent results of old Hungarian turkey crossbreds.

Keywords: Old Hungarian turkey, Copper turkey, Bronze turkey, meat quality, meat production

Broiler genetic strain affects breast meat quality, histology, white striping incidence and gene expression

Servet Yalcin1, Mustafa Akşit2, Guldehen Bilgen3, Bulent Helda, Sene Orkun4, Gamze Turgay Izzetoglu4

Ege University, 1Faculty of Agriculture, Department of Animal Science, 2Faculty of Science, Department of Biology, Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, 3Cine Vocational School, Turkey

This study was conducted to determine meat quality, muscle histology, the incidence of white striping and expression of IGF-1 and myogenin in breast muscle of fast-growing broilers from two commercial genetic strains. A total of 200 chicks from Cobb and Ross broilers (100 from each strain) obtained from a breeder stock aged 38 wk were reared in the same poultry house using standard broiler-type feed. The live weight was measured from week 18 to 24 weekly and at the slaughterhouse. The birds were slaughtered at the age of 28 weeks and the weight of the individual pieces of carcass (breast, leg) was measured. The following meat quality parameters were investigated in breast: colour, drip loss, pH, electrical conductivity, kitchen technology losses, shear force value. At week 24, Converter hybrid x Bronze had the highest live weight (14.22 kg), Converter hybrid x Copper and Bronze x Converter groups reached the same weight (12.99 kg, 12.14 kg), the lowest live weight was measured in the Copper x Converter group (10.02 kg). The Converter hybrid x Copper had the lightest (L=52.49), but the Copper x Converter genotype had the reddest (a*=4.32) breast meat. The favourable drip loss was in the Converter x Copper (6%) and Converter x Bronze (6.7%) groups. As a conclusion, constructions with the paternal line produced the highest results in both production and meat quality. From these, Converter hybrid sire x Bronze dam genotype had more favourable meat production compared to the Converter hybrid sire x Copper dam genotype. On this basis, if the breeding goal is directed to a larger carcass, it is worthwhile to use a meat hybrid paternal line for better results. Further breeding and testing are recommended because of excellent results of old Hungarian turkey crossbreds.

Keywords: Old Hungarian turkey, Copper turkey, Bronze turkey, meat quality, meat production

Effect of lighting program on carcass features and meat quality in broilers

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The aim of this study was to investigate the effects of four different lighting programs on some slaughtering, carcass and meat quality characteristics of broiler chickens. The first group was continuously applied to 23 hours of light (23L) and 1 hour of darkness (1D) which is widely used in the broiler production. The second group was 4L:2D, 4L:2D, 4L:2D; the third group was 8L:4D; the fourth group was continuously 16L:5D. A total of 500 male Ross 308 chicks were used for this purpose. The chicks were divided into 4 experimental groups with 5 replications, each replicate having 25 birds, with equal starting weights. The research lasted 42 days. At the end of the experiment, chickens in each replicate were weighed and, 10 chickens close to the group average were chosen. A total of 40 chickens were slaughtered under suitable conditions. The breast meat pH was measured 10 min after slaughtering. M. pectoralis profundus was taken in the slaughtered broilers and kept at -20 °C until analysis. When the results were analyzed, slaughter (P<0.01), carcass (P<0.01) and breast weights (P<0.001) were lower in the fourth group and, crude fat and protein content were higher in this group (P<0.01). The protein content was higher in the second group than the other groups (P<0.05). The pH value of breast meat was observed highest in the first group (P<0.001). The effect of lighting program on white syndrome was not determined (P>0.05). According to the results of this study, 16 hours of light and 8 hours of dark program (Group 4) resulted in negative effects. As an alternative to the continuous lighting program commonly used in poultry husbandry in today’s sector, it has been determined that short-term dark programs will be more suitable in terms of the examined features.

Keywords: Broiler, lighting program, carcass features, meat composition, white line syndrome

Causes of carcass condemnations of broiler chickens at industrial slaughterhouse of Ardabil, Northwest of Iran

Aldin Arzoo1

Associate Professor of Poultry Diseases, University of Mohaghegh Ardabili, Ardabil, Iran

The aim of the present study was to determine causes of carcass condemnations of broiler flocks at Ardabil poultry abattoir, Northwest of Iran. The data were collected by observation from May 2015 to March 2016. Total number of carcasses, total number condemned and number of those condemned for disease and pathological changes were recorded. In the study period, 3,650,751 birds were slaughtered. Out of these, 46971 (1.28%) carcasses were slaughtered. Different reasons were identified for carcass condemnations. Condemnation causes and its percent of total number condemned including: septicemia (36.5%), poisoning (22.1%), dead on arrival (9.3%), ascaris and pentonitis (8.9%), cachexia (7.8%), chronic respiratory disease (6.3%), overscaling (4.7%), synovitis and arthritis (2.8%), contamination (1.2%) and bruises (0.4%). Septicemia and poisoning were the most common reasons for rejection of carcasses and were responsible for 58.6% and 0.75% of the total condemnations and the total slaughter, respectively. The results of this study showed that the most frequent reason of condemnations are caused by diseases which also are important for public health. Therefore, improving methods of prevention and control diseases on farms is necessary.

Keywords: Abattoir, Condemnation, Broilers, Ardabil

The meat production of Yellow Hungarian Chicken breed in different keeping systems

Árpád Drobnyák1, Mónika Heininger1, Károly Kustos1, László Bődi1, Rubina Tündé Szabó1, Szonja Cesge Skrbá1, István Szalay1, Ágnes Zimborán1, Mária Weber1

1Szent István University, Faculty of Agricultural and Environmental Sciences, Institute of Animal Husbandry, Research Centre for Farm Animal Gene Conservation (HáGK), 2Research Centre for Farm Animal Gene Conservation (HáGK); Association of Hungarian Small Animal Breeders for Gene Conservation, 3Lab-nyúl Kft., Szent István University, Faculty of Agricultural and Environmental Sciences, Institute of Animal Husbandry

The meat production of Yellow Hungarian Chicken breed in different keeping systems
Specific activity of intestinal enzymes in response to immediate and delayed access to feed and water in broiler chickens

Reza Mirbakhsh, Shirin Horoberk, Seyed Davood Shariati, Seyed Aminzadeh

This experiment was conducted to investigate the effects of different levels of post-hatch feeding with hydrated diet supplemented with probiotic and transport tensions (including: shipping temperature (24°C and 30°C) and shipping time (12, 24 and 48 hours)) from hatchery to poultry house, on specific activity of duodenum enzymes (amylase, aminopeptidase and lipase). Seven hundred and twenty newly hatched male broiler chickens (Ross 308) were distributed in a completely randomized design into 12 treatments (1: 24°C × 12 h× 0 (g gel/bird), 2: 24°C × 12 h× 1 (g gel/bird), 3: 24°C× 24 × 0 (g gel/bird), 4: 24°C× 24 × 2 (g gel/bird), 5: 24°C × 48 × 0 (g gel/bird), 6: 24°C× 48 × 4 (g gel/bird), 7: 30°C× 12 × 0 (g gel/bird), 8: 30°C× 12 × 2.5 (g gel/bird), 9: 30°C× 24 × 0 (g gel/bird), 10: 30°C× 24 × 2.5 (g gel/bird), 11: 30°C× 48 × 0 (g gel/bird), 12: 30°C× 48 × 5.25 (g gel/bird) with 3 replicates and 20 birds per replicate, during 39-d. At the end of 7 days of age, an observation per replicate were slaughtered to determine specific activity (Units/mg of tissue protein) of amylase, aminopeptidase and lipase in samples of homogenated duodenum. According to the results, feeding status in treatments which were slaughtered at different time and shipping temperature, had no significant differences. Specific activity of aminopeptidase was different between 12 treatments (P<0.05) but there were no significant difference was observed for amylase and also lipase. Comparing the results of treatments no. 1, 3 and 5 explains the effect of shipping time on specific activity of enzymes. In mentioned treatments, delayed access to feed and water in birds which were transferred in 24°C, did not affect the activity of amylase and lipase (P>0.05), but aminopeptidase activity was decreased (P<0.05). The same trend was observed for birds with immediate access to feed and water which were transferred in 30°C (treatments 7, 9 and 11). Comparing the results of treatments no. 1, 3 and 5 with 7, 9 and 11 showed that increasing shipping temperature from 24°C to 30°C did not affect specific activity of amylase and lipase (P>0.05), in birds which had the same time of feed and water deprivation. According to the results, the effect of shipping temperature on specific activity of duodenum enzymes, was more important than feeding status. Therefore, the suitable temperature during transport of chickens from hatchery to poultry house should be taken into account specially in long distances.

Keywords: Amylase, Aminopeptidase, Lipase, Post-hatch feeding

Study of the prevalence of Salmonella in poultry slaughterhouses and cutting plants

Hang Zeng, Koen De Reu, Sarah Gabriel, Lieven De Zutter, Geertreui Rasschaert

Salmonella is considered as the most common cause of foodborne outbreak bacterial pathogen. Most of the human salmonellosis cases are attributed to meat products and eggs. To prevent Salmonella in poultry industry, the Salmonella status of broiler flocks is determined before slaughter in view of logistic slaughter in the EU. This means that flocks with a Salmonella free status are slaughtered first each day followed by flocks with a positive status. However, this can only be efficient when the slaughter line and equipment before starting slaughter activities is Salmonella free and thus cleaned and disinfected efficiently. This study was conducted for helping the participating poultry slaughterhouses to eliminate Salmonella contamination. The study aimed to investigate the possible contamination of the first slaughtered flock by sampling the equipment before starting slaughter activities. A thorough sampling in the production environment of 3 broiler and 2 laying hen slaughterhouses was performed. Different swab and water samples were taken from the hanging area, the scalding tank, the plucking machine, the evisceration machines and the cutting line. Each slaughterhouse was visited twice. Salmonella detection on the samples was based on the ISO 6579 standard. The presumptive colonies were confirmed by Salmonella PCR. From the 652 samples of the slaughter equipment collected after cleaning and disinfection in the 5 slaughterhouses 43 (6.6%) were positive for Salmonella. The prevalence of Salmonella in slaughterhouses A, B, C, D, E was 24/136, 4/134, 8/130, 3/128, 4/124 respectively. Among the 5 sampling stages, the results show that the plucking machine (26/150) was the most contaminated machine, especially the plucking fingers. This finding helps the elimination of Salmonella in the poultry slaughterhouse, especially preventing Salmonellosis contamination at the start of the slaughter activities.

Keywords: poultry slaughterhouses, poultry cutting plants, Salmonella

Enhancing the technological quality of processed poultry meat products by using plant-based ingredients that are rich in dietary fiber in formulation matters of interest to the researchers. In this line, this study aimed to investigate the effects of utilization of pumpkin powder in batter formulations of chicken nuggets on technological characteristics. For this purpose, batters were formulated with 100% wheat flour (C), 50% wheat flour + 50% pumpkin powder (W/P) and 100% pumpkin powder (P). Chicken nuggets were produced by incising chicken breast muscles, preparing the nugget dough by addition of salt, molding, pre-dusting with wheat flour, battering with one of the three formulations mentioned above, coating with breadcrumbs, and finally deep-fat frying. Water holding capacity, adhesion ratio, cooking yield and oil absorption were analyzed to evaluate the technological quality of the nuggets. Incorporation of pumpkin powder in batter formulations negatively affected the water holding capacity with respect to the amount of pumpkin powder used. It was observed that the use of pumpkin powder increased the adhesion ratio of batters compared to the use of wheat flour in formulation. Cooking yields of W/P and P samples were similar to C samples, and increased concentrations of pumpkin powder led increment in cooking yield. No significant differences were recorded in oil absorption among the treatments. As a conclusion, this study demonstrated that pumpkin powder could be a promising ingredient as wheat flour replacer in batter formulations for chicken nuggets without sacrificing most of the technological quality parameters.

Keywords: wheat flour, pumpkin powder, chicken nugget, technological quality

Technological quality of chicken nuggets formulated with pumpkin powder as wheat flour replacers

Burcu Öztürk Kerimoğlu, Hülya Serpil Kavuşan, Melih Sarıcalı, Dilara Kış, Meltem Serdaroğlu

This finding helps the elimination of Salmonella in the poultry slaughterhouse, especially preventing Salmonellosis contamination at the start of the slaughter activities.

Keywords: poultry slaughterhouses, poultry cutting plants, Salmonella
The relationship between air deformation test and meat quality traits of woody breast fillets

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Woody breast (WB) is characterized by a distinct hardness of raw fillets which can vary in severity. This condition has been emerging problem in the poultry industry. Developing methods to identify WB potentially online would be valuable to the industry in efforts to sort product. This study was conducted to determine effectiveness of air deformation of fillet surfaces to identify WB in raw fillets and the meat quality of cooked fillets.

Fillets (n=90) were collected from raw fillets, CF increased as severity of WB increased (P<0.05, NORM-MOD=SEV). Diameter measurements of air deformation decreased as WB categories increased regardless of distance (D12, D15, or D18). CF was highly correlated to D15 (r=-0.80) and D18 (r=-0.76). Cook loss was higher in SEV fillets compared to NORM and MOD fillets (P<0.05). There were differences (P<0.05) in SEV fillets due to WB categories, but BOMORE was increased (P<0.05) as WB categories increased. SEV fillets had higher (P<0.05) PC-MORS and NORM fillets whereas PC-BMORS increased (P<0.05) as severity of WB increased. The results indicate that SEV WB negatively affect cook loss and shear properties. CF can be identified by CF and the use of air deformation in raw fillets may be potentially used as tool for online detection.

Gapping of pectoralis minor muscles: an emerging quality issue for broiler processors?

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Recently, some broiler abattoirs have signaled the occurrence of an emerging quality issue termed as “gapping” because of the separation of the fiber bundles affecting the external surface of the Pectoralis minor muscle. Thus, being the first investigation carried out on this topic, the present study aimed at defining proper criteria to classify the muscles as normal, moderate (MOD) and severe (SEV) and subsequently assess the incidence rate of the gapping defect under commercial conditions. The surveys were carried out in a major Italian processing plant on a total of 8,600 chickens (from 42 to 54 day-old, average live weight of 2.84 kg) randomly chosen from 43 flocks during a six-month period. In order to establish the criteria to classify as MOD and SEV cases, both the number and the length of the fiber bundle separation points were considered. In detail, the muscles exhibiting a distance (D12, D15, or D18). CF was highly correlated to D15 (r=-0.80) and D18 (r=-0.76). Cook loss was higher in SEV fillets compared to NORM and MOD fillets (P<0.05). There were differences (P<0.05) in SEV fillets due to WB categories, but BOMORE was increased (P<0.05) as WB categories increased. SEV fillets had higher (P<0.05) PC-MORS and NORM fillets whereas PC-BMORS increased (P<0.05) as severity of WB increased. The results indicate that SEV WB negatively affect cook loss and shear properties. CF can be identified by CF and the use of air deformation in raw fillets may be potentially used as tool for online detection.
The meat production of pigeon varieties and the benefits of the pigeon meat are known. However, the objective indices of pigeon meat have not been studied in this system to the present in the literature. Our aims were to investigate the objective quality parameters for pigeon meat. Our further aim was to produce a protocol that allows the qualitative evaluation of pigeon meat and starts to establish the standardization of the test protocol. We recognised King (K) and Texan (T), the two current, similarly high-performance varieties based on body, carcass and breast meat weight. Moreover, colour, pH, electrical conductivity, kitchen losses (baking and cooking losses) and tenderness (share force value) of the breast meat were analysed. However, based on the data available in the literature, the Kings’ (weight 764±16 g) were found higher, but the Texans were somewhat smaller (711±33), even though the difference was not significant (p=0.2). On the other hand, the difference in the carcass (K: 490±2 g; T: 470±9 g; p=0.045) and the breast meat (K: 136±8 g; T: 115±7 g; p=0.028) was significant. This meant we were able to examine birds that are considered to be average, so we tried the samples we had already tested on broilers on suitable samples (e.g. square based column with 1x1 cm edge length; Warner-Bratzler share force value). Additionally, comparing the King and Texan varieties, we did not find any significant differences in the majority of the parameters despite the difference in weights. There were no large or statistically justified differences between the rest of the species even though an example was found in other species of poultry. In the light of all these it can be said, that the meat investigation protocol for broilers can be used for pigeons as well, but further measurements are needed to establish an adequately stable standardised system. On the other hand, we have to involve other types of meat and/or other varieties, which broaden the possibility of exploring potential critical points. Dissemination of the above results is necessary, as we analysed parameters for pigeon breast meat, which has not been done to the present in the literature. However, this can help the breeders’ work in reaching breeding objectives in the context of meat quality. This work can contribute/enable consumers to be properly informed and will lead to the popularization of pigeon meat.

**Keywords:** pigeon, breast, meat quality

### Electrical stunning of poultry: influence of animal sex and weight

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Stunning in slaughterhouse is an essential stage before bleeding and allowing to maintain the animal unconscious until its death. This practice is under the regulation EC 1099/2009. In France, electrical water bath stunning is the most widespread method to stun broiler chickens, for which the regulation imposes a minimum current to be distributed to each chicken, depending on the frequency applied. According to the literature, the unconsciousness of animals is best guaranteed for applied frequencies below 200 Hz and an applied intensity per animal above 120 mA, while products quality is better for frequencies above 1000 Hz and an intensity below 100 mA. However, parameters other than the electrical parameters may influence stunning efficiency and the carcasses quality such as sex and weight of animals. The sex of animals did not have significant effect on stunning efficiency regardless of the parameters tested. However, for the electrical parameter 90mA / 400Hz, females presented more red heads than males. The weight of animals influenced stunning efficiency and carcass quality. Regardless of the electrical parameters, heavy chickens (2.5 kg) had fewer severe class red wingtips than those in other weight categories. Furthermore, with the parameter 90mA / 400Hz, the heavy chickens after bleeding, shook more the head and the wings. With the electrical parameters 150mA / 400Hz, the chickens were all influenced stunning efficiency and carcass quality. Regardless of the electrical parameters applied, heavy chickens (2.5 kg) had smaller muscular exercise in an open air area covered with vegetation. The aim is to investigate the applicability of these indexes using commercial non-experimental samples as a validation group. Twenty chicken busts, half of which labeled “slow growing”, were purchased in commercial food markets. The busts were randomly divided into 5 groups of 4 T. The impulse variables were 30 °C at 1200 Hz and 30 °C at 1000 Hz, respectively (20.54±1.31 g; 30.57±1.31 g). There were no significant differences in fat content between the two types of samples because of high variability of data, but comparing the glycolytic fibers from the oxidative muscle were not statistically significant differences in the parameters: 3.5 and 6.6 desaturase were found on “slow growing” labeled chickens (p<0.001). The same differences (about 60%) were recorded by comparing the amino acids of the breast and thigh. Therefore by applying discriminant analysis to validate the indexes, only one animal has not been classified correctly. The tested indexes could therefore be used to identify commercial hybrids with an exploratory behavior from those without.

**Keywords:** indexies for slow growing, lipid and metabolism, chicken behaviour

### Optimizing of the gut health in broilers received yeast ((Saccharomyces cerevisiae) combined with threonine in diets

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**Abstract**

In intensive avian production systems, as broilers, a healthy gastrointestinal tract is essential for improving conversion index by the efficient assimilation and absorption of nutrients, probiotics and antibiotics. The antagonistic action against pathogens (Saccharomyces cerevisiae) combined with threonine in diets has proved to be a protective performance. Threonine has an important role in ensuring the intestine optimal function. The aim of this research was to evaluate the association of Saccharomyces cerevisiae (Sc) with Threonine (T) in broiler diets, on performance, carcass quality and intestinal health. Seventy-five Ross male broilers (14-42 days old) were distributed into three treatments: D1: commercial type, D2: plus 0.5 % Sc and 30 % T, D3: D1plus 1 % Sc and 30 % T. Each treatment group of 25 chicks was randomly subdivided into five subgroups (replicates) comprised of five chicks each. Broilers received a starter diet (14 to 28 day old) and finisher. Gain (g/ broiler/ day), Average Daily Consumption Feed (g/broiler/day) and Feed Conversion Ratio. At the end of the experiment, broilers were slaughtered to determine carcasses quality from each broiler as Breast, Thigh and Abdominal Fat Weight (AFW) (g). Then, were taken-off gut samples and processed by conventional histological technique and stained with PASβ to determinate goblet cells and plasmatic cells (IgA producer) number/villi area, mucus layer size and measure Villus Height/ Crypt Depth (V/H/C) ratio. The dates were analysed by ANOVA and the LSD test. Performance productive and carcass quality were similar between all treatments except AFW, where D3 group had less fat than D2 and D1 groups (p<0.05) (28.59±0.8, 26.07±0.51, 28.76±0.7, respectively). In gut, goblet cell number/villi area was higher in D3 (2.39±0.30) than D2 (2.29±0.12) and D1 (2.12±0.35) (p<0.005) and plasmatic cells/villi area was higher in D3 (0.87±0.10) than D2 (0.49±0.09) and D1 (0.39±0.09) (p<0.05). Mucus layer was higher in D3 (2.05±0.10) than D2 (1.67±0.26) and D1 (1.55±0.31) (p<0.05) and V/H/C ratio were lesser in D3 (4.26±0.2) respect to D2 (5.31±0.8) and D1 (5.26±0.4) (p<0.05). Conclusion: Sc associate with T produced broilers with better carcass quality through less lipid abdominal. Also, Sc combined with T increased healthy gut, that could be produced by more mature epithelia that enhancing absorptive function through the efficient use of nutrient and increasing protective function through mucosal hypersecretion by increased goblet cell number and plasmatic cells (IgA producers).

**Keywords:** poultry, gastrointestinal system, threonine, probiotics, antibiotic, broiler.
Keywords: S. cerevisiae –Threonine - Gut Health – Broilers - Nutrition.

Introduction

In intensive avian production systems, as broilers, a healthy gastrointestinal tract is essential for improving conversion index by the efficient nutrient utilization. It is fundamental into the first weeks of life, where the growth and developed of gut succeed, the microbiota colonizes the gut and interact with intestine and Gut-associated immune system (GALT). So, the broilers production is looking for natural additives alternatives to Antibiotics Growth Promotor (APG) used 20-30 years ago, because the international and national regulations have banned their use off in the feed. Also, the increased development and spread of antibiotic resistance in a microorganism and the possible presence of antibiotic residues in poultry products have contributed to looking for broilers free antibiotic products. In this way, arise different natural nutrients know as probiotics, prebiotics, phytotherapeutic, oil vegetal, etc. used in intensive productions, as Avicultura (2). For example, yeast (Saccharomyces cerevisiae), probiotic and prebiotic, is a source of proteins, fibre, minerals, provides essential B vitamins and organic acids (3), resulted in increased growth weight and improved health in broilers (3-7).

The positive effects of Sc on performance (feed conversion) and carcass quality of broilers fed with yeast alone 0.3-1% or replacing 1/3 of the premix (0.5-1 g/Kg) were proved in broilers diets (8,9).

Threonine (T), an essential amino acid is requested in broilers body protein synthesis and body maintenance. Also, it is found in the gastrointestinal epithelium and as a component of immunoglobulin molecules, so it is important for intestinal health and overall digestive processes and under pathological conditions (sepsis, for example) increase T requirement to maintain intestinal mucosal integrity (10-13).

Although there are experiences adding T or Sc in broiler diets, there are a few essays adding Sc plus T (our previous research). Taking account of this absent, the aim of this research was to evaluate the association of Saccharomyces cerevisiae (Sc) with Threonine (T) in broiler diets, on performance, carcass quality, and intestinal health.

Materials and Methods

Seventy-five Ross male broilers (14-42 days old) were housed in pens, in Avian Research University, in Rio Cuarto National University (RCNU). All animal handling and experimental procedures were approved by Bio-Ethics Committee RCNU. All chicks were weighed on day 14 and distributed randomly into three treatments: D1: commercial type, D2: D1 plus 0.5 % Sc and 30 % T, D3: D1 plus 1 % Sc and 30 % T. Each treatment group of 25 chicks was randomly subdivided into five subgroups (replicates) comprised of five chicks each. Broilers received a starter (14-28 day old) and finisher a 28-42 day old. Feed and water offered ad libitum. Diets were formulated according to (13). Sc was used powder whole, dehydrated (Virgen®) and it was T-L-Threonine (Ajinomoto®).

Productive variables: during the experimental period, initial (Day 14) and final (Day 42) Weight total broiler/each pen were obtained. Also, all feed used was registered to measure Consumption. Broilers mortality was recorded and percentage mortality was determined at the end of the assay. Performance productive parameters measured were: Average Daily Weight Gain (ADGW) (g/bird/day), Average Daily Consumption (ADC) (g/bird/day) and Feed Conversion Ratio (FCR) (1).

Carcass quality: at 42 days old, weight from each broiler in each pen was taken, chicken were slaughtered to determine carcasses quality. From each broiler, was removed and weighted breast, thigh and abdominal fat. The variables measured were: Breast and Thighs Weight (BW and TW, respectively) (g/broiler) and Abdominal Fat Weight (AFW) (g/broiler) (1).

Gut health: two chicken from each pen were selected randomly to obtain gut samples for histopathological and histomorphometric variables. Samples of 2 x 2 cm of the middle ileal segment between Meckel’s diverticulum and the ileoceocal junction were taken, fixed immediately in buffer formalin and processed following the histologic classic Technical, stained with PAS with histopathological examination by optical microscopy (OM) (Axioskop- Carl Zeiss, Germany) with a digital camera [Powershot G6, 7.1 megapixels (Canon INC, [Japan]) attached to OM was used. It was determine goblet cells and plasmatic cells (IgA producer) number/villi area, mucus layer size and Villus Height/Crypt Deep Ratio (VH/CD ratio). The VH/CD ratio was processed with the software AxioVision V 4.6.3 (Carl Zeiss, Germany), taking a minimum of 20 fields per histological section (2).

The dates were analysed by ANOVA and the LSD test, using Infostat software® (14). p≤0.05 were considerate significate.

Results

Performance productive and carcass quality were similar between all treatments except AFW, where D3 group had less fat than D2 and D1 groups (p=0.05) (20.59±4.8; 26.17±5.01; 28.76±7.57, respectively).

In the gut, goblet cell number/villi area was higher in D3 than D2 and D1 (p=0.005) and plasmatic cells/villi area was higher in D3 than D2 and D1 (p=0.05). Mucus layer size was higher in D3 than D2 and D1 (p=0.05) and VH/CD ratio was lesser in D3 respect to D2 and D1 (p=0.05) (Table 1 and Figure 1).

Table 1: Gut Histomorphometric variables in broilers fed yeast (S. cerevisiae) and Threonine.

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goblet cell number/villi area</td>
<td>2.12±0.35 a</td>
<td>2.29±0.12 a</td>
<td>2.89±0.30 b</td>
</tr>
<tr>
<td>Plasmatic cells/villi area</td>
<td>0.39±0.09 a</td>
<td>0.49±0.49 a</td>
<td>0.87±0.10 b</td>
</tr>
<tr>
<td>Mucus layer size (µ)</td>
<td>1.55±0.31 a</td>
<td>1.67±0.26 a</td>
<td>2.05±0.10 b</td>
</tr>
<tr>
<td>Villus Height/Crypt Deep Ratio</td>
<td>2.56±0.4 a</td>
<td>5.31±0.8 a</td>
<td>4.26±0.2 b</td>
</tr>
</tbody>
</table>

Table: D1: commercial type, D2: D1 plus 0.5 % Sc and 30 % T, D3: D1 plus 1 % Sc and 30 % T. a, b, different letters mean significative different p< 0.05

Figure 1: Intestinal Villi. Intestinal Histopathology Microphotography in D1 and D3 Group (Figure 1 and II, respectively. Into D1 group, is noticed a few Goblet Cells(a) and Mucus, and some plasmatic cells (IgA producers) (b) inside the villi. In D3 group, is noticed abundant Goblet Cells (a) and Mucus, and abundant plasmatic cells (IgA producers) (b). Stained with PAS-40 X.

Discussion

Nowadays, the gastrointestinal tract is studied as a dynamic environment, with interactions between enterocytes, leucocytes, goblet cells and the content of the gastrointestinal lumen (non-nutrient dietary factors, nutrients, and microbiota). These interactions determine the degree to which nutrients are digested and absorbed, the level of immune activation in the gastrointestinal tract, the food and environment host for secreting and synthesizing mucin, and other factors that contribute to the animals’ health and productive performance (2).

Natural additives can influence the development of healthy gut flora, intestinal wall, and immune defense. Also, T levels are essential for gut development and physiology that is reflex in a healthy gut, too (1,11,12).

In the present research, the productive variables (ADC, ADWG, and FCR) of broilers were not affected significantly by Sc and T addition, although ADC and ADWG were 3 % higher in D2 respect to D1 and D3 (no significate), FCR was similar between all broilers. About Carcass Performance, there was a tendency to higher BW and TW in D3 (no significate) and 25 % less AFW respect to D1 and D2 (p<0.05). Also, it did not register mortality during the experience. In general, fed Sc combined with T increased gut health, through better use of nutrient (positive effect of Sc) and better muscle deposition (positive effect of T) with better meat quality through lower abdominal fat in D3 group, as the consumers prefer.

According partially to this result, in a previous research we found that Sc (0.5 %) combined with different levels of T (15-45%) produced similar performance productive in broilers of 43 days old but BW and TW increased and AFW diminished in the groups had received higher levels of T (30 and 45 %) (1, 2). In another research, not register any changes in carcass quality of broilers fed Sc (0.5 %) combined with T (0-7.5 %) (10).

Gut health, registered by histopathology observations and histomorphometric variables, was significantly better in D3 group respect to D2 and D1 (Table 1). In D3 group, VH/CD was 20 % less than the other groups, because of increased absorption area, it is by T addition, which generate that D3 broilers could take better advantage of the nutrients. Also, the CD was increased in D3, it means that these broilers had increased turnover for a rapid immune response when potentially damaging pathogens contact with the intestines cell exchange (7, 11, 12).

Also, goblet cell number and mucus layer size was approximately 50 % higher in D3 with respect to D1 and D2 and was noticed 44 % increased the plasmatic cells number with respect to another group.

According to the results, in the previous essay, we noticed better HV/CD in broiler groups receive T alone (30 %) or combined with Sc (at the same level used on this essay) in the diet (1,2). In another experience, it was registered deeper crypts in broiler received the addition of T alone, although the level was lower (2.5-7.5 g/Kg feed).
Both Sc and T are associated to healthy gut through a different mechanism: Sc induce to lengthened villus, so it is associated with improved nutrient absorption and increases the activity of enzymes secreted from the tip of villi resulting in improved digestibility. Also, cell wall components of Sc provide a protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigen to be in contact with the villi. Different researches affirm that taller villi indicate more mature epithelia and enhanced absorptive function due to the increase absorptive area of the villi (7, 12).

Contrarily to this result, did not register changes in gut health parameters (VIH and DC) in broilers fed outer cell wall of Sc (glucomannan-protein complex) or Sc (1.5 g/kg feed) (7, 12). Perhaps, the different nature of Sc (total) or wall cell Sc interact with microbiota and GALT by a different mechanism to our assay, modify the balance between gut, microbiota, and immunity but not influence gut histomorphometry parameters in the broilers (1, 2).

On this essay, together with the decrease, VIH/DC Ratio increased the protection on the villi layer and gut mucosa by higher mucus layer produced by increased goblet cells number in D3. This mucus layer is first line of broiler defence against invading pathogens and help with transportation of different substances between the lumen and the epithelial cells (7). Also, mucus layer contains IgA, which found increased in the D3 group. IgA regulates the ecological balance of microbiota and mucus homeostasis (1,2), so Sc combined with T provide a great advantage for broilers to a greater elimination of intestinal pathogens and therefore an improved protection system against intestinal infections (1, 2, 7). This increase in gut health parameters results in few better carcass performance, because higher villi produced by Sc plus T, increase nutrient absorption and increased mucus layer protection and mucosal gut immunity perhaps modify the interactions microbiota – gut and immunology cells.

Conclusion: Sc associate with T produced broilers with better carcass quality through less fat abdominal. Also, Sc combined with T increased healthy gut, that could be produced by more mature epithelia that enhancing absorptive function through the efficient use of nutrient and increasing protective function through mucosal hypersecretion by increased goblet cell number and plasmatic cells (IgA producers).

Acknowledgment

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References


Antioxidant effect of E vitamin in broiler breast meat at different times post slaughter

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The aim of this study was to analyze the effect of vitamin E, at different concentrations in the diet, on the lipid oxidation of broilers breast meat at different times post slaughter. Eighty broilers (28-50 days old) were divided into: C (Control), V1 (C + 50 ppm Vitamin E), V2 (C + 100 ppm Vitamin E) and V3 (C + 200 ppm Vitamin E), with four replications of five birds each. Breast samples were taken from the carcasses and stored at -22°C. They were analyzed by thiobarbituric acid method (TBARS), at three times post slaughter: one, five, and ten days. ANOVA and post-hoc Tukey tests were done, p> 0.05 were considered significant. The breast samples from V1 and V2 had significantly lower TBARS values than C and V3 (2.90 nmol/g ± 0.11; 2.845 nmol/g ± 0.12; 7.85 nmol/g ± 0.21 and 4.083 nmol/g ± 0.16, respectively) until the fifth-day post slaughter. At 10 days, the values increased and were similar in all groups. We conclude that E vitamin in the diet could control lipid oxidation in meat until 5 days post slaughter. Also, V1 and V2 showed a better antioxidant lipid effect than V3 at this time.

Keywords: Vitamin E, Lipid oxidation, Antioxidant, Breast Meat

Reducing Campylobacter contamination via a probiotic feed supplement

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3Maaza, Bacillus, Probiotics, Campylobacter, B-Act®

Abdominal seeder birds were added on day 21. After 16 extra days (d37) the caecal samples were taken from the carcasses and stored at -2/2 ºC. They were analyzed by thio-barbituric acid (TBA) method as follows: a group receiving no probiotic supplementation, a group supplemented with 1.6 x 10^9 CFU Bacillus licheniformis (B. licheniformis)/gram of feed, a group supplemented with 3.2 x 10^9 CFU B. licheniformis/gram of feed and a group supplemented with 6.4 x 10^9 CFU B. licheniformis/gram of feed. Each group started with 20 Campylobacter spp.-free birds, to which 20 Campylobacter spp. positive seeders were added on day 21. After 16 extra days (d37) the caecal content of 10 non-seeder birds per group was sampled and the concentration of Campylobacter spp. (in log10 CFU/g caecal content) was determined, by inoculating decimal dilutions on Campylobacter spp. specific agar plates.Compared to the control group B-Act® fed at 6.4 x 10^9 CFU/g of feed significantly (P<0.05) reduced Campylobacter concentration in the caecum of broilers: 6.55 vs. 4.75 respectively, in log10 CFU/g caecal content. B-Act® supplementation at lower levels reduced Campylobacter numerically, with values of 6.01 and 5.59 respectively (for 1.6 (P<0.05) reduced Campylobacter concentration in the caeca of broilers: 6.55 vs. 4.75 respectively, in log10 CFU/g caecal content. B-Act® significantly reduced the Campylobacter spp. colonization of the caeca. Dosages were mainly lower in caeca of birds supplemented with higher dosages of B-Act®, resulting in a significant dose-response effect being observed. As such, B-Act® can contribute to food safety and the reduction of zoonotic bacteria in poultry production.

Keywords: Bacillus licheniformis, B-Act®, Probiotics, Campylobacter, Food safety
Effect of pumpkin seed meal on physicochemical and sensory traits of broiler meat

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The aim of this research was to determine the effect of pumpkin seed meal (PSM) on physicochemical and sensory traits of broiler meat. For that purpose, 360 male one-day old chickens (Ross 308) were allocated to three different groups (P-0, P-5 and P-10) including four replications. The diet for P-0 group contained 0% of PSM, while diets for P-5 and P-10 groups contained 5% and 10% of PSM, respectively. After feeding period of 42 days, broilers were slaughtered, and analysis of physicochemical and sensory traits were performed on 10 randomly chosen breast muscles from each treatment. The average pH values ranged between 5.73 and 5.77. The average values of lightness L* were from 61.79 to 62.63, redness a* from 12.87 to 3.90, and yellowness b* from 14.25 to 14.75. The average drip losses were between 2.32% and 2.47%, while average cooking losses were between 15.53% and 17.70%. The average TABK values of abdominal fat ranged between 0.432 and 0.595. No statistically significant differences were found between treatments except in TABK value, which was the lowest in P-10 group. Sensory analysis did not reveal effect of addition of PSM in feed on smell, taste or juiciness of breast meat. Therefore, it can be concluded that addition up to 10% of PSM in broiler feed does not have any adverse effect on meat quality.

Keywords: broiler, meat quality, pumpkin seed meal, sensory traits, TABK

Dose response of a phytase on (phytate) phosphor, protein and ash digestibility and on bone ash in broilers

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in crude protein and crude ash digestibility. Adding the phytase (except for T5), significantly increased the total P digestibility (72.5-74.8%) compared to the PC (55.8%). Concerning the phytate P digestibility, all groups had a significantly higher digestibility (range 57.6-66.1%) compared to the PC (41.1%). Compared to the NC (57.6%) only the two highest doses (T7 and T8; 74.3% and 81.1%, respectively) were significantly higher. The tibia crude ash concentration was significantly higher for all treatments (range 39.5-45.4%) compared to the NC (36.3%), where the addition of the two highest phytase doses (T7 and T8; 43.6% and 43.9%, respectively) no longer significantly differed from the PC (45.4%). Based on these results, adding the phytase at 1250 and 1500 FTU/kg could compensate for the decrease in available phosphorous.

Keywords: broiler, digestibility, phytase

Effect of new generation plant extract mixture on quail performance and caecal microflora

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In this study, effects of dietary supplementation with new generation plant extract mixture on performance (body weight gain, feed intake and feed conversion ratio) and caecal microflora of quails were investigated. A total of 300 1-day-old Coturnix coturnix specimens were divided into four groups containing 75-quails and treated as follows: (1) a control group with 0 g Filopower/ton of diet-C, (2) 100 g/ton Filopower/ton of diet-G1, (3) 150 g/ton Filopower/ton of diet-G2 and (4) 200 g/ton Filopower/ton of diet-G3. The Filopower used as a natural growth promotion, in the quail and support the immune system. Additives were used at the rate of 1% of the diet. The treatments were allocated to a randomized complete block design. All treatments were equal in shipping conditions and compared with the control group. Salmonella spp. were not detected in caecal samples. As a result, new generation plant extract mixture is an antibiotic which has a natural, performance-enhancing, no residual risk, the healthy digestive tract potential, anti-inflammatory effect and support the immune system in the quail rations. Because of these properties, it was concluded that the use of the filopower additive in the quail rations could have beneficial effects.

Keywords: Quail, plant extract mixture, caecal microflora, Lactobacillus spp, Salmonella spp

Effect of post-hatch feeding with hydrated diet supplemented with multi-nutrients on performance of broiler chicken

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Dehydration of one-day-old chickens while carrying them from hatchery to farm is one of the problems in the poultry industry. Dehydration will increase the bird’s mortality, loss of body weight, slowdown of absorbing yolk, reducing feed intake, reducing weight gain, lowering body temperature, and less resistance to heat, cold, and also weakening the immune system of the birds. This experiment was conducted to investigate the effects of post-hatch feeding with hydrated diet supplemented with multi-nutrients (water, soya, groundnut, dicalcium phosphate, sodium chloride, L-lysine, methionine, vitamin A, vitamin D3, vitamin B1, vitamin B2, niacin, pantothenic acid, vitamin B6, vitamin B12, biotin, vitamin E, vitamin K, vitamin C, choline, capric acid, MOS, beta-glucan, bifidobacterium bifidum, lactobacillus acidophilus, lactobacillus bulgaricus, lactobacillus casei, lactobacillus plantarum, streptococcus thermophilus, streptococcus thermophilus, aspergillus oryzae, torulosip spp, cellulase, protease, xylanase, beta-glucanase, alpha-amylase, pectinase, lipase, phytase, dextrane) on adjustment of transport tensions (including: shipping temperature (34°C) and shipping period (4, 16 and 28 hours) of chickens from hatchery to farm, on performance of broiler chicks during 42 days. A number of 480 newly hatched male broiler chickens (Ross 308) randomly divided into 6 treatments (1-4 hours × 0 g Feed/ton, 2-4 hours × 2 g (gr Feed/ton), 3: 6 hours × 0 (gr Feed/ton), 4: 16 hours × 2 (gr Feed/ton), 5: 28 hours × 0 (gr Feed/ton), 6: 28 hours × 2 (gr Feed/ton)) with 4 replicates and 20 birds per replicate. Performance (feed intake, weight gain and FCR) was studied at 7, 14, 21, 35 and 42 days of age. Results showed that performance of chickens which were fed hydrated diet supplemented with multi-nutrients, during different shipping times was not statistically significant. Feed intake of the chicken, increased with deprivation time (P = 0.047). In conclusion, the bycic ingredients could support the chicks during 28 hours shipping period.

Keywords: Hydrated diet, Shipping time, water and feed deprivation

Effect of fasting and early nutrition on morphology and microflora of intestine in broiler chickens

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An experiment was conducted to investigate the effects of different levels of early nutrition (hydrated diet supplemented with probiotic) and shipping conditions (shipping temperature (24°C and 30°C) and shipping time (12, 24 and 48 hours) from hatchery to poultry house, morphological development of jejunum (jejunal length, jejunal width, jejunal surface area), intestinal villus height, villus width, crypt depth, number of goblet cells and number of ileal bacteria). A total of 300 1-day-old Coturnix coturnix species broiler chicks divided into 10 treatment groups (T1- T10) were used in this study. The birds were allocated to 10 experimental groups containing 30 birds each. The birds of the control group (NC) were transferred at 30°C. Comparing treatments 1, 3, 5 with 7, 9, 11 showed that shipping temperatures in birds which had deprivation time of 12, 24 and 48 hours had no significant morphological changes. Same results were observed for birds with no access to water and feed which were shipped at 30°C. Comparing the results of treatments 1, 2, 4, 5 and 8; 2, 5 and 8; 2, 5 and 10; 2, 5, 8, 9 and 11 showed no significant difference in morphological parameters. According to the results of treatments which were same in shipping conditions but different in feeding status, population of Coliform (P=0.061), Lactobacillus (P=0.411) and bifidobacterium (P=0.867) had no significant differences. Effect of shipping time was not significant for bacterial population in intestine (treatments 1, 5, 9). The same results was obtained by increasing shipping time for treatments 7, 9,11 which had deprivation and transferred at 30°C. Comparing treatments 1, 5, 9, 11 showed that increasing shipping temperature from 24°C to 30°C had no significant effect on bacterial population. It is concluded that effects of post-hatch feeding on morphological status and microflora population of intestine, appear to be mainly short-term.

Keywords: Bifidobacterium, Coliform, Lactobacillus, Lamina propria

Identification and denomenclature of some species of migratory birds vectors of avian influenza viruses

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Migratory birds, especially ducks (order Anseriformes), constitute the primary reservoir and first source of introduction of the avian influenza virus (Al). In 2016, following the first official detection of the circulation of the subtype H9N2 in broiler farming’s in Eastern Algeria, a preliminary descriptive study is conducted to identify and enumerate in the humid areas of the Eastern Highlands of Algeria, the species migratory birds, particularly Anseriformes and Charadriiformes, known to carry Al. In the order Anseriformes, 9 species of the family Anitidae are identified, 6 are known infected: Mallard (Anas platyrhynchos, Mallard); Winter Teal (Anas crecca crecca, Common Teal); Pintail (Anas acuta, Northern pintail); Gadwall (Anas strepera); Eurasian Wigeon (Anas penelope) and Common Shelduck (Tadorna tadorna). In order of Charadriiformes, 2 species of the family Laridae, are identified, of which only 1 species is infected: Black-headed Gull (Larus ridibundus). In the order Pelecaniformes, only 1 species of the family Phalacrocoracidae, was detected: Great Cormorant (Phalacrocorax carbo), and is known infected. Our observations increase the diversity of migratory bird species, particularly Anitidae that are infected with the Al, in the humid areas of Eastern Algeria. Molecular characterization of circulating avian influenza viruses in these wild birds will make it possible to assess the risk of a probable spread of these viruses to populations of intensive poultry farming’s.

Keywords: Migratory birds, Al, humid areas, Algeria
Oxidative and Microbiological Quality of Phosphate-Free Restructured Chicken Steaks During Frozen Storage

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Abstract
Phosphate salts are one of the mostly used additives in meat products to improve functional features of proteins, besides they act as antioxidants and indirect antimicrobials. However, due to the tendency towards healthier meat products, today use of clean-label ingredients in formulation of meat products has come into prominence. The present work aimed to investigate the oxidative and microbiological quality of frozen restructured chicken steaks produced with inulin (either as powder or gel form) with/without sodium carbonate as sodium tripolyphosphate (STPP) replacers. Chicken breast cubes were tumbled with sodium chloride and STPP for production of control treatments. Other treatments were formulated with sodium carbonate, inulin in powder form, inulin+sodium carbonate in powder form, gelled inulin or gelled inulin+sodium carbonate as STPP replacers. During the frozen storage of 5 months at 18°C, analyses were carried out to evaluate the lipid oxidation (TBARS value), protein oxidation (total carbonyl content) and microbial load (total aerobic mesophilic bacteria, Enterobacteriaceae, yeast-mold counts). The initial TBARS values of the samples were between 0.210-0.558 mg malonaldehyde/kg, while the values were between 0.902-1.238 mg malonaldehyde/kg at the end of the storage. Microbial load significantly increased over storage time by the formation of secondary oxidation products, the results were mostly similar among the formulations. At the beginning of the storage, the total carbonyl content of samples ranged between 0.843-0.885 mmol/mg. Increment in carbonyl content of the treatments was significant at the end of the storage, which was between 0.952-1.061 mmol/mg. Yet all the phosphate-free samples had similar carbonyl content with control samples at 5th month. The total aerobic mesophilic bacteria count was similar among the samples, that was between 2.69-3.43 log CFU/g, while at 5th month it was between 3.05-3.23 log CFU/g, indicating that there was only a slight change in the microbial load by the effect of low temperature. At all analyse periods, Enterobacteriaceae and yeast-mold counts were lower than 100 CFU/g. Our results showed that utilization of inulin in restructured chicken steaks showed equivalent quality parameters in terms of lipid oxidation, protein oxidation, and microbiological stability during frozen storage, regardless of usage form and combination with sodium carbonate.

Keywords: Phosphate, inulin, carbonate, restructured chicken steak, frozen storage

Effects of using broccoli powder and sodium carbonate as phosphate replacers on the quality characteristics of model system chicken emulsions

Oxidative and Microbiological Quality of Phosphate-Free Restructured Chicken Steaks During Frozen Storage

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Materials and methods
Model system chicken emulsion was prepared according to the method described by (Hurtado et al. 2012) with modifications. Four different chicken emulsions were formulated: In control samples 0.5% sodium tripolyphosphate was added, whereas in the three experimental batches 0.2% of the addition of phosphates on consumer health. Recently, a high phosphate concentration has also been found to be an independent predictor of cardiovascular events and mortality in the general population. Therefore, phosphate additives in food are a matter of concern, and their potential impact on health may well have been underestimated (Ritz et al. 2018). A study of patients in stage CKD 5 (with an annual mortality of about 20%). Recent research has revealed that consumers tend to choose natural sources of functional ingredients rather than chemical additives, and that they will pay significant premiums of 100% or more for natural ingredients (Carocho et al. 2015). Moreover, the meat processing industry has already taken steps to find suitable alternatives to synthetic additives, such as nitrates and ascorbic acids, to meet consumer demands (Jang et al. 2017). Thus, there is a requirement for finding natural ingredients for the replacement of phosphates (E450) (Choe et al. 2016). Broccoli (Brassica oleracea L. var. Italica) is recognized as a functional food due to its high content of health-promoting compounds such as ascorbic acid, phenolic compounds, glucosinolates and isothiocyanates (Aguilar-Camacho et al. 2019). Broccoli has enormous nutritional and medicinal values due to its high contents of vitamins (A, B1, B2, B6, E), minerals (Ca, Mg, Zn, and Fe) and antioxidant substances which prevent the formation of cancer causing agents (Gid and Abd El-Moe 2017). Broccoli is an alkaline vegetable with a pH of 6.3-6.9 so in this investigate when it is considered broccoli will low cooking losses and high the water holding capacity.

Table 1 Chemical composition is K, C, B and BC groups.

| GRUPLAR(Urun) | Nem | Protein | Yag | Kil | Dret | Ta | ETP | Gr | K | C | B | BC |
|---------------|-----|---------|-----|-----|------|----|-----|----|----|----|----|----|----|
| A 70.538±0.0635 | 18.311±0.5611 | 5.028±0.2172 | 2.908±0.0028 |
| B 70.261±0.0726 | 17.724±1.1058 | 4.3446±0.3462 | 2.6634±0.3794 |
| C 67.656±1.5825 | 20.097±0.3331 | 8.6322±0.4400 | 2.447±0.1931 |
| BC 70.393±1.3418 | 18.530±0.3328 | 7.9615±2.8234 | 2.8724±0.0901 |

References
- Ritz et al. 2018
- Carocho et al. 2015
- Long et al. 2014
- Choe et al. 2016
- Aguilar-Camacho et al. 2019
- Carocho et al. 2015
- Becker 2012
- Flynn and Bramblett 2006
- Murphy 1975
- DUMAS method with a LECO nitrogen analyzer (FP-528, USA)
- pH was measured from three different points by using a pH-meter (WTW pH 336, Germany)
- Color parameters were measured with a portable colorimeter (Konica Minolta, CR-200, Japan)
- Cooking yield (Murphy et al. 1975), water holding capacity (Hughes et al. 1997) and emulsion stability (ES) as total expressible fluid (TEF) and the expressible fat (EFAT) and were analyzed.

Results
The pH of CE without phosphate (B) was the lowest among all treatments and the addition of Carbonate significantly increased its pH (p < 0.05, figure 1). The CE with the addition of 1% broccoli powder+0.2% sodium carbonate (BC) had a higher pH value than that of the group with phosphates. Samples added with broccoli powder + sodium carbonate (BC) showed higher fat retention values than other samples. This results was showed that the high pH of the C and BC groups would have increased its water holding capacity, owing to the decreased the exudation of fat and water. There were no differences in water hold capacity between the K group and C group; however, water hold capacity decreased between the B and BC groups. The B groups showed the highest released water, higher than that of the other groups. Consequently, when no phosphate added, there were significantly decreases on water hold capacity of chicken emulsions. However, with phosphate additions, the increase on water hold capacity of emulsions. TBARS values of CE samples, presented in Fig. 2, were significantly affected by formulation and storage time (p<0.05). The values were between 1.02-1.13 mg malonaldehyde/kg at the beginning of storage. Initial values were nearly similar to each other K, BC and B samples and higher TBARS values than C sample (p<0.05). At the end of the storage period (day 6) lowest TBARS values of the samples were in BC samples which were 1.05 mg malonaldehyde/kg. This result might be a good indicator that Broccoli had an advantage to delay lipid oxidation, due to the phenolic compounds, and it could present equivalent behavior to phosphates acting as an antioxidant agent.
Figure 1 pH values on CE during the storage.

Figure 2 TBARS values on CE during the storage.

Discussion

Broccoli powder and/or sodium carbonate was studied as an alternative to phosphates in model system chicken emulsions. The addition of 0.2% C to sausages inhibited the exudation of fat from sausages and increased the pH of meat batter. Similar results were obtained by Öztürk and Serdaroğlu (Lipid oxidation phenomena leads to negative effects on the quality of meat and meat products in terms of sensory attributes and nutritional value, as well as on health (Nunez et al., 2008)). TBARS value is an indicator of secondary products formed in fatty acid oxidation chain reactions. In addition, lipid oxidation of sausages was inhibited with the addition of 1.2% BC (1.0% broccoli powder+0.2% sodium carbonate). Therefore, this research indicates that sodium carbonate can effectively replace phosphate in meat emulsions and utilization of broccoli in combination with carbonate had promising effects as phosphate replacers by providing equivalent quality to standard phosphate containing products.

References


Composition of diet matrix in broilers are being altered according to their stage of production, which is carried out by change in ingredients composition. Use of animal protein sources especially fish meal has been increased. As the search for an alternative to antibiotics continues, the polyphenols (PP) are now being considered as a candidate. In addition to its variability in quantity and magnitude polyphenols, exhibit varied results due to difference in nutritional matrix. The present experiment was carried out to study how the change in protein matrix with alternative cereal source (rice-sorghum instead of corn as cereal source) and level of polyphenols alters the meat lips and sensory characteristics. A total of 160 broiler chicks which were divided into four groups and reared for 42d under hot-humid environment (Temp: 29-36°C; RH: 69-80%). G1: Rice-sorghum-soybean-50ppm PP; G2: Rice-sorghum-soybean-50ppm PP without PP; G3: Rice-sorghum-soybean-50ppm PP and G4: Rice-sorghum-soybean-fish meal diet +100ppm PP. The polyphenols used in this experiment was extracted from pomegranate peels using methanol as solvent. The muscle cholesterol and triglycerides levels were significantly decreased (P<0.05) in PP (G1, G3 and G4) supplemented groups when compared to negative control (G2). The muscle thiosulphuric acid reactive substances (TBARS) was significantly (P<0.05) higher in G2 as compared to other three groups. The muscle carotenoid content showed changes due to age of supplementation. The supplementation of PP significantly (P<0.05) increased the muscle carotenoid content in G1, G3 and G4 when compared to G2 at 42d of age. The carotenoid content was comparable (P>0.05) among the groups at 28d of age. The meat characteristics such as flavour and overall acceptability was affected by the fish meal fed groups (G2, G3 and G4 vs. G1). The supplementation of PP to these groups (G3 and G4) significantly (P<0.05) improved their flavour and overall acceptability but some was still lower than the soybean (G1) alone as protein source fed group (G1). Other parameters related to sensory quality of meat such as colour, juiciness and tenderness on a one to eight point scale were comparable (P>0.05) among the treatments. From this it could be concluded that, the incorporation of fish meal significantly affect the flavour and overall acceptability of broiler meat, however, the supplementation of polyphenols improved muscle quality and acceptability.

**Keywords:** Broilers, carotenoids, lipid content, polyphenols, protein matrix, TBARS

**Comparative analysis of the microbiome associated to poultry carcasses from conventional and antibiotic free farms**

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The aim of this preliminary study was to compare the microbiome of poultry carcasses reared in conventional (N=5) and antibiotic free (N=5) farms. An aliquot of neck and breast skin was obtained from each individual carcass at the end of the refrigeration tunnel and submitted to DNA extraction. Total DNA was then shotgun sequenced and reads analysed using MG-RAST to classify the colonising bacteria up to the species level and to check the presence of antibiotic resistance genes. Bacteria belonging to the genera Acinetobacter, Chlorobium, Propionibacterium, Psychrobacter and Rhodococcus characterized the microbiome of poultry carcasses from broilers reared in antibiotic free farms. Furthermore, bacteria belonging to the genera Anoxybacillus, Escherichia, Lactobacillus and Pseudomonas characterized the microbiome of poultry carcasses from broilers reared in conventional farms. In terms of antibiotic resistance genes, the microbiome associated to poultry carcasses from broilers reared in antibiotic free farms did not display genes coding for resistance to fluoroquinolones, methicillin, streptothricin, beta lactam and aminoglycoside detected in the microbiome of carcasses from broilers reared in conventional farms.

**Keywords:** microbiome, shotgun sequencing, antibiotic free farm, conventional farm

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**Polyphenols improved the meat lipid and sensory attributes affected by incorporation of fish meal as alternate protein source in broiler chicken diet**

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The mixture homogeneity is an issue of serious concern in the course of adding of an insignificant amount of feed components to the mixture and poultry feed homogeneity. Mixter coefficient of variation is the most important parameter in poultry feed homogeneity. So, to accurately calculate the coefficient of variation, correct marker selection is very important and it affects the accuracy of the evaluation. Zinc is a candidate marker that can be used for calculation of mixer coefficient of variation. An experiment conducted to evaluate two sources of Zinc oxide, for scrutinizing mixer efficiency and poultry feed homogeneity. Zinc sources included potentiated ZnO, (HiZoxTM) and regular ZnO, both contained 76% pure Zn. Physical characteristics, like particle size, flowability, cohesiveness with two different sources were different. A completely randomized design was performed with two treatments (ZnO sources) each in ten replicates. Trial was done in the laboratory feed mill of Academy of Minatoyoor. All conditions (mixer type, mixing time, added zinc) were the same. Samples were taken by special sampling instrument from the discharge of three-dimensional paddle turbo mixer. Zinc content of broiler breeder premix samples was measured by atomic absorption spectroscopy. Results indicated that homogeneity of premix affected by the source of zinc oxide (P<0.01). The coefficient of variation for premix contained potentiated zinc oxide was significantly better than those added by regular one (5.65 vs 5.65). In conclusion, regardless of the source of nutrient markers, physical properties like particle size and defined shape, can affect poultry feed homogeneity and manufacture equipment evaluation.

**Keywords:** poultry feed, quality, evaluation, zinc

**Evaluation of the effects of activated zinc oxide on the specification of broiler chicken bone**

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Nine hundred male broiler chicken were used to evaluate the effect of activated zinc oxide at different dosages on the specification of broiler chicken bone. A completely randomized design was used with three treatments. Treatment replicated ten times and 30 bird in each. Basal corn-soybean meal diets formulated for starter, grower and finisher periods supplemented with 75, 100 and 125 mg/kg zinc from HiZox® (76% pure zinc) for making three treatments. After slaughter the left tibia of birds removed in order to the measurement of dimensional parameters and minerals content (ash, phosphorous, calcium and zinc). Bone resistance to breaking force was determined by the mechanical instrument. Treatments had no significant effect on tibia weight, length and diameter. No difference was observed in tibia breaking strength at peak force pressure. The calcium and zinc content of the bone was not affected by dietary zinc content. Birds received 125 mg/kg added zinc showed significantly higher bone phosphorus content than those fed 75 and 100 mg/kg added zinc diet (P<0.05). However, tibia breaking strength, included elongation and extension, improved by consumption of diet supplemented with 75 mg/kg HiZox® (P<0.05). The results of the present study also suggest that 75 mg/kg zinc from HiZox® can improve tibia properties in broiler chickens.

**Keywords:** Broiler, HiZox, Tibia, breaking strength

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**Scrutinizing mixer efficiency and poultry feed homogeneity**

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The mixture homogeneity is an issue of serious concern in the course of adding of an insignificant amount of feed components to the mixture and poultry feed homogeneity. Mixter coefficient of variation is the most important parameter in poultry feed homogeneity. So, to accurately calculate the coefficient of variation, correct marker selection is very important and it affects the accuracy of the evaluation. Zinc is a candidate marker that can be used for calculation of mixer coefficient of variation. An experiment conducted to evaluate two sources of Zinc oxide, for scrutinizing mixer efficiency and poultry feed homogeneity. Zinc sources included potentiated ZnO, (HiZoxTM) and regular ZnO, both contained 76% pure Zn. Physical characteristics, like particle size, flowability, cohesiveness with two different sources were different. A completely randomized design was performed with two treatments (ZnO sources) each in ten replicates. Trial was done in the laboratory feed mill of Academy of Minatoyoor. All conditions (mixer type, mixing time, added zinc) were the same. Samples were taken by special sampling instrument from the discharge of three-dimensional paddle turbo mixer. Zinc content of broiler breeder premix samples was measured by atomic absorption spectroscopy. Results indicated that homogeneity of premix affected by the source of zinc oxide (P<0.01). The coefficient of variation for premix contained potentiated zinc oxide was significantly better than those added by regular one (5.65 vs 5.65). In conclusion, regardless of the source of nutrient markers, physical properties like particle size and defined shape, can affect poultry feed homogeneity and manufacture equipment evaluation.

**Keywords:** poultry feed, quality, evaluation, zinc
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