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Advances in Poultry Nutrition Research

Edited by Amlan Kumar Patra

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Meet the editor

Amlan Kumar Patra earned his Ph.D. in Animal Nutrition from the Indian Veterinary Research Institute, India, in 2004. He has worked at West Bengal University of Animal and Fishery Sciences as an assistant professor since 2007. He also worked at the American Institute for Goat Research, USA (2004–2006); the Ohio State University, Columbus, USA (2010–2012); and the Free University of Berlin, Germany (2017–2018). His research has focused on animal nutrition (particularly ruminants and poultry nutrition), gastrointestinal electrophysiology, meta-analysis and modeling in nutrition, and livestock-environment interaction. He has authored approximately 160 articles for professional journals and various book chapters and proceedings, resulting in a Google Scholar h-index of 40 with over 6800 citations. He currently serves as editor and associate editor on the editorial boards of various reputable journals.

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Preface

Most of the cost in poultry farming involves feeding management. Therefore, the profitability of poultry farming depends on a precisely formulated diet that provides all the essential nutrients in proper proportions. Because poultry diets consist primarily of corn and soybeans, the cost of such diets is high due to the high price of these crops. Therefore, it is essential to use other low-cost feed ingredients in poultry diets. These low-cost ingredients need to be properly proportioned so that production performance is not hampered.

Chapter 1 discusses research advancements in poultry nutrition. Chapters 2 and 3 present nontraditional poultry feed ingredients, including microbial hydrolyzed feather protein, which is useful in replacing costly protein ingredients. The evaluation of protein sources in various quail feed formulations is discussed in Chapter 4. Mineral excretion is one of the major issues in livestock and poultry farming, due to the overuse of supplements to fulfill mineral requirements. Minerals from common supplements are only absorbed to a limited extent. Minerals from nanomineral sources are better absorbed through the intestine and thus their inclusion in diets may be reduced, which can also reduce environmental burdens and the cost of feed. The topics of mineral supplements and their use are highlighted in Chapter 5. In Chapter 6, we discuss the effects of environmental temperature and how it affects poultry water intake.

Apart from production aspects, poultry health is crucial to reduce mortality and to prevent several diseases including coccidiosis, wooden breast myopathies, and gut disorders. Proper nutritional management can reduce these issues. The use of antibiotic feed additives is common in the poultry industry, but concerns have been raised regarding the potential for antimicrobial resistance, which has compelled the restriction or exclusion of antibiotics in poultry feed. This has in turn led to the use of alternative feed additives such as essential oils, flavonoid compounds, probiotics, prebiotics, synbiotics, nanoparticles, and antimicrobial peptides.

Chapter 7 delineates the importance of poultry gut health and immunity in production. Chapter 8 discusses the role of secretory defense in the gut for maintaining poultry health. The usefulness and limitations of probiotics in balancing the gut's microbial populations, nutrient utilization, and immunity are discussed in Chapter 9. Coccidiosis caused by the Eimeria spp parasite is one of the major diseases affecting the health and performance of poultry reared in intensive production systems. Chapter 10 points out how coccidiosis can be managed through appropriate nutritional strategies. Today's fast-growing broiler chickens show an increase in skeletal muscle abnormalities such as white striping and wooden myopathies characterized by degeneration, necrosis, and atrophy of muscle fibers. This is a great concern in broiler meat production resulting in the need to discard chicken meat. Chapter 11 highlights the occurrence, prevention strategies, and economic losses due to these abnormalities. Poultry experience heat stress occasionally throughout the year, especially in developing countries where poultry housing is not mechanized to maintain temperature. Heat stress exerts adverse effects on production, reproduction, gut health, and nutrient utilization, which may reduce the profitability of poultry production. Chapter 12 details how heat stress impacts production variables and how it could be alleviated by dietary means.

Taken as a whole, this book covers a wide area of poultry nutritional issues including the utilization of nontraditional feeds and nanominerals, materials that can economize feed formulation, the importance of gut health, and gut-health maintenance through the use of probiotics. This publication includes chapters on common poultry diseases such as coccidiosis, white striping, and wooden breast myopathies, and the effect of heat stress on production, reproduction, and poultry health. The editor hopes the information offered here will be useful to industry researchers and experts to improve their knowledge and understanding of important advances in poultry nutrition.

The Editor of this book would like to thank each of the authors for their valuable contributions. The Editor would also like to thank Ms. Maja Bozicevic for her excellent communication with the authors and editor during the editorial process of this book.

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Section 1

Nutrition and Production

Chapter 1

Advances in Poultry Nutrition Research-A Review

Herbert Kwabla Dei

Abstract

Poultry production involving farmed avian species (chickens, turkeys and ducks) is globally recognised as a vital aspect of animal agriculture. It contributes greatly to supply of high-quality protein (meat and egg) for humans. The intensification and commercialization of the poultry sector is accelerated and continues to be so as a result of research discoveries in the field of breeding, nutrition, housing management and disease control. However, the explosion in poultry nutrition research, in particular, has significant impact on the success of the poultry sector. The progress in nutritional research is made possible by several advanced techniques reported by researchers in both academia and industry. Nutritional research is mainly geared towards improving knowledge on ingredients good for the growth and health of the bird and of nutritional requirements for various types and classes of birds, as well as the ability to match nutritional requirements of any type or class of bird for variable conditions including that of the environment. This chapter discusses the importance and growth of the poultry sector. Also, nutritional research efforts over the years, achievements, some notable advance research techniques employed, and challenges confronting nutritional research in the 21st Century have been highlighted.

Keywords: poultry, nutrition, research, advanced techniques, review

1. Introduction

Globally, poultry (avian species such as chickens, turkeys, ducks and guinea fowls) production is an important aspect of the animal agriculture. It is undeniable fact that the intensification and commercialization of the poultry sector is accelerated by research discoveries in the field of breeding, nutrition, housing management and disease control. That is to say, the success of the poultry sector is underpinned by considerable research efforts over the years through application of scientific innovations. These research efforts were largely geared towards the following: improving genetic strains specialised for food use (meat and eggs) and regional conditions; improving knowledge of nutritional requirements and ability to match these for variable conditions; and ensuring stable environment for growth and production. These research efforts are still ongoing in the light of new challenges facing the animal industry in terms of birds' welfare and issues of environmental pollution as well as consumers' concerns of food quality and safety.

So far, the objectives of nutritional research include selecting ingredients good for the growth of the bird, enabling conditions for the bird to express its full genetic potential, eliminating certain disease conditions, reducing the cost of production, maintaining product quality and allowing for partial alleviation of the adverse effects of environmental factors. Thus, advances in nutrition research are crucial if the poulry sector is to continue to play a major role in animal protein food production in meeting the needs of ever-increasing world population.

This paper discusses the importance and growth of the poultry sector as well as highlights the nutritional research efforts over the years, achievements, some notable advance research techniques employed, and challenges confronting nutritional research in the 21st Century.

2. Importance and growth of the poultry sector

Poultry meat and eggs are cherished worldwide and consumed in various forms. They are proteins and a source of essential micro-nutrients such as vitamin A, vitamin B_{12}, riboflavin, calcium, iron and zinc [1]. Therefore, they are important in human nutrition and health.

Both the meat and eggs are produced in large quantities all over the world for food and income. As such, the poultry sector is one of the leading suppliers of meat worldwide [2]. So much income can be derived from poultry products. For example, America which is the world's leading producer of poultry in 2017, had its combined value of poultry meat and eggs up to the amount of $42.7 billion [3]. Besides, poultry products are generally cheap, making them affordable for low-income earners in both developing and advanced countries.

In fact, the poultry sector has the potential to grow faster as a result of enabling factors such as population growth, breeding of highly-productive strains of meat- and egg-type birds, improvements in consumers' incomes, and modern technologies for processing feed/poultry products. However, the cost of feeding poultry is a major factor controlling the push for more incomes by farmers [4].

3. Overview of poultry nutrition research and development

Research involving avian species such as chickens, turkeys and ducks in the early 1900's appeared to be limited in scope (e.g. native birds) and unstructured. However, by the middle of 1900's, there began a surge in research with a structured approach in all aspects of production with greater focus on nutrition [5]. Nutritional science is one that looks at the biological sum of processes that occur before and after food intake as they relate to growth, development and health maintenance [6]. Thus, nutritional science relies very much on the other sciences (chemistry, physics, immunology, biochemistry, behavioural sciences, physiology, microbiology, molecular biology, statistics, genetics and food science) and often studied in an integrated way; since it is not a pure science but draws extensively on these root disciplines. Also, nutrition is complex; therefore, nutrition research requires sophisticated designs and analytic capabilities that can be addressed in an interdisciplinary and multidisciplinary manner.

In terms of progress made in poultry production, it has been reported that research in genetics contributed more than nutrition. For instance, genetics accounted for 85–90% of the change in growth rate of broilers and turkeys over the past 50 years, whereas 10–15% was attributed to advances in nutrition and management [5]. **Table 1** [7] shows some dramatic successes chalked over the years through poultry genetic research. However, the realisation of the genetic potential of birds,

Avian specie	Trait	Performance level		
		1960	2005	Δ (%)
Broiler chicken	Number of days until 2 kg	100	40	**60**
	Kg feed per kg live weight	3.0	1.7	**43**
Layer chicken	Number of eggs per year	230	300	**30**
	Number of eggs per tonne of feed	5,000	9,000	**80**

Table 1.
Increase of genetic potential with consequent superior efficiency of poultry production.

increased productivity, and decreased susceptibity to disease depend on appropriate nutrition. Hence the relevance of nutritional research.

The phenomenal progress in the poultry sector brought about by scientific researches is achieved through the concerted efforts of researchers in both academia and industry. The academic community comprises centres of higher learning such as universities and national research institutions, while the industrial community consists of the private sector including commercial breeding and development companies. The academic community is primarily involved in basic research by exploring new grounds, which enable building of a storehouse of pertinent knowledge; and applied research for direct application to the industry; whereas the industrial community takes their experimental procedures into the realm of industrial application to build a business.

The nutritional research focus in both academia and industrial sector over the years has been changing in response to challenges that faced poultry production in terms of output, economic, environmental and consumer demands. The nutritional research focus in the 1950's to 1980's was on production efficiency with the whole animal in mind; and then, there was a shift in research focus between 1990's and today to maximisation of biological and economical performance with regards to the whole animal or selected organs and tissues [5]. Also, there has been more integration of nutrition with other disciplines such as microbiology and nano technology. In fact, prevailing challenges in the poultry sector suggest that future research focus will be more about efficiency of meat/egg production, meat/egg quality and safety for human consumption, feed efficiency to reduce environmental pollution and health and welfare of birds [5].

Some spectacular research successes in nutrition have been documented [4, 5] as shown in **Table 2**.

Research Period	Achievements
1900–1910	• Emergence of the notion of a 'vitamin' to define secondary dietary factors • Discovery of Vitamin and its relationship with carotene
1910–1920	• Discovery of calcium and phosphorus requirements for the young chick and laying hen • Distinction between water and fat soluble Vitamin A • Lysine recognised as being essential to the growth of young chicks
1920–1930	• Vitamin D recognised as the active factor in cod liver oil • Existence of many vitamins within the B group postulated

Research Period	Achievements
1930–1940	• Discovery of riboflavin a the active factor in milk products necessary to prevent the condition of 'curled toe' in young growing chicks • Concept of calcium: phosphorus balance • Discovery of vitamin K • Perosis caused by manganese deficiency • Isolation of vitamin B1 (thiamine) • Initiation of work on improvements to soya bean meal and its use in animal feeding • Discovery of threonine as the latest essential amino acid • Development of the use of fishmeal • Isolation of pantothenic acid • Synthesis of pyridoxine (vitamin B6) • Sale of synthetic riboflavin, niacin, vitamin E, vitamin K, choline and biotin
1940–1950	• Evidence of biotin deficiency • First definitions of biotin requirements of poultry • Development of pelleting of feeds • First tables from National Research Council (NRC) • Estimations of vitamins and amino acids through microbiological techniques • Sale of synthetic methionine • Discovery of folic acid • Sale of synthetic vitamin A • Appearance of first effective coccidiostats • Formulation of energy rich diets for broilers • Discovery of cyanocobalamin (vitamin B12) previously referred to as 'animal protein factor'
1950–1960	• Development of Near Infrared Reflectance Spectroscopy (NIRS) for feed analysis • Vitamin and mineral requirements • Nutrient content of feedstuffs • First use of antibiotics in poultry production • General use of synthetic methionine • First use of analogue computers in diet formulation on the basis of 'least cost' (optimisation routines) • Discovery of zinc as a trace element for poultry • First commercial sale of lysine of industrial origin • General use of the concept of metaboilsable energy in poultry production
1960–1970	• Amino acid content of feedstuffs • Dietary amino acid requirements • First use of linear programmes for diet formulation on personal computers • First commercial sale dwarf breeders

Research Period	Achievements
1970–1980	• Refinement of nutrient requirements in terms of their digestibility in feedstuffs • Identification of mycotoxins and their dietary tolerance limits • Development of feed additive: antibiotics, growth promoters and coccidiostats • Prediction equations for requirements • Modelling of requirements of poultry • Development of true digestibility in poultry • Nutrition of secondary species (turkey, guinea-fowl, duck) • Routine use of NIRS for the rapid analysis of raw materials
1980–1990	• Computerised least-cost feed formulation • Understanding anti-nutritional factors in feedstuffs • Use of supplemental enzymes • Development of the ideal protein concept and formulation for digestible nutrients, and the absorption and utilisation of nutrients • Commercial sale of synthetic tryptophan and threonine
1990s until today	• Use of statistical packages (SAS, GENSTAT, etc) to ease computational burden of large data and accurate estimation of production performance, growth, and feed consumption • Complex modelling for biological and economic performance • State-of-the-art technologies for weighing feed and birds, feed evaluation, meat processing, etc. • Non-intrusive ultrasound technology to assess breast meat yield of live bird • Lixiscope that uses x-ray to safely detect and identify Tibia Dyschondroplasia (TD) • New and advanced microscopy techniques

Table 2.
Nutritional research achievements through the years.

4. Advances in nutritional research

Over the years, researchers have improved or introduced new research techniques in their quest to obtain valid data or keep pace with new challenges confronting the poultry sector. In this section, I will attempt to discuss some of the advances made in poultry nutritional research with the aim of improving productivity and quality of meat/eggs, welfare of birds as well as environmental sustainability. These include modern techniques for feed analysis and nutritional experimentation involving novel feed ingredients and/or feed additives, nano-minerals, mineral toxicity as well as dietary management strategies to curtail problems and constraints of avian health and environment.

4.1 Feed analysis

The surest way of meeting the nutrient requirements of birds is having adequate knowledge of nutrient contents of various feedstuffs available. This requires feed analysis with regards to nutrient composition and anti-nutrient contents. There have been advanced techniques employed over the years which give true reflections of chemical composition of feedstuffs that enables nutritionists to be able to accurately formulate diets for all types and classes of poultry. These include improved methods for determination of dry matter, proteins, carbohydrates including fibre, fats/oils and macro-and micro-minerals as contained in the official Methods of Analysis (2019) and published by the Association of Analytical Chemists (AOAC) International [8]. These techniques not only determine the true content of feedstuffs but also saves time and amounts of samples to be analysed as well as minimum operator training. Examples are vacuum-oven drying/toluene distillation of high fat feeds for moisture determination; Automatic Kjeldahl Analyser/Dumas technique (LECO)/Amino Acid Analyser for protein determination; Megazyme enzyme kit for starch determination in cereals; Rose-Gottlieb/Soxflo method for fat determination; Fibre Analyzers for determination of acid detergent and neutral detergent fibres, and the use of Inductively coupled plasma optical emission spectrometry (ICP-OES) analyser for determination of several minerals at the same time; Near-infrared spectroscopy (NIRS) technique for determination of moisture, crude protein, metabolisable energy and digestible amino acids of whole feed sample [8].

The presence of anti-nutritional factors (ANFs) in feedstuffs compromise the potential nutritional value of feedstuffs for poultry. Some have detrimental effects on the health of birds. Thus, identification and quantification of anti-nutrients in feedstuffs through advances made in feed analysis has improved nutritional value of hitherto inedible plant products into useful feedstuffs. For instance, the identification and subsequent elimination of trypsin inhibitors in soybean improved its feed value for poultry substantially. Examples of analytical methods for detecting ANFs are as follows: Yb-precipitation/Vanillin-HCl/4-dimethylamino-cinnamaldehyde (DMACA-HCl)/BSA/PEG/mass spectrometry methods for determination of tannins; High-performance liquid chromatography (HPLC)/Acid–base titration/acid dye colorimetry for determination of alkaloids; and ELISA methods for determination of mycotoxins [8].

4.2 Feed formulation

Feed formulation is crucial in meeting nutrient requirements of poultry. This aims at avoiding excess nutrient supply as much as nutrient deficiency. Advances in this regard are made possible by computer software developers. Prominent among poultry feed formulation software is the Least-Cost formulation software. It is helpful in formulating high-quality diets for different types and classes of birds at a low cost. Generally, feed cost constitutes more than 60% of poultry production cost [9]. Therefore, use of this advance technique in diet formulation greatly reduce the cost of feeding birds; which may account for affordability of poultry products.

The most valuable advance technique in poultry nutrition is the use of the NIRS technology [10–12]. Apart from accurately predicting the chemical composition of raw feed ingredients and feeds, this technology enables the farmer to rapidly measure metabolizable energy (ME) and digestible amino acid (DAA) in real time (or near real time) for major feed ingredients [13–15]. ME and DAA are the main dietary components considered in poultry feed formulation. The actual ME and DAA contents of feed ingredients (e.g. different geographical locations or batches) could vary substantially from the specifications setup (matrix) in the feed formulation software leading to formulation errors. This technology is used to enhance precision feed formulation in relation to ME and DAA. The results can be obtained immediately after NIRS scanning of feed ingredients. Although NIRS equipment is expensive, all other costs to implement the technology can be reduced.

4.3 Feed utilisation

Effective means for dietary feed to be quantified and prepared is to perform studies of digestibility and bird growth efficiency studies. This helps in feed quality assessments. Advances made with respect to determination of feed utilisation are ever-evolving. These include: Growth assay technique, *In vivo* and *In vitro* digestibility techniques, Inert marker technique, Caecetomy and Ileotomy techniques and Ileal assay technique [16–19]. The advantages of these techniques among others are high precision data, reduction in duration and cost of experimentation, less labour-intensive, use of small feed samples, avoid use of live birds for experiments, and rapid routine feed quality assessments.

4.4 Use of novel feed ingredients

Traditionally birds are grain feeders; however, with advent of industrial processing of food for humans, a lot of by-products have been generated and research extensively for poultry feeding [20–22]. These include cereal offals, oilseed meals, brewer's dried grains, distillers dried grain with solubles (DDGS), etc. Besides, through research, lesser used feedstuffs such as barley, rye, sorghum, cassava and grain legumes are increasingly being processed for feeding [23–28]. Furthermore, other plant resources in the wild such as false yam [29] and forages [30] are being harnessed for feeding due to advances in feed processing technologies and analytical tools. The essence of using these novel feed ingredients is to serve as alternatives for conventional feed ingredients such as maize (*Zea mays*) and animal proteins (e.g. fishmeal, meat and bone meal). This is aimed at reducing feed cost or curtailing dependency on these conventional feeds, particularly in developing economies.

4.5 Use of feed additives

Feed additives are employed in poultry diets in order to improve digestive efficiency. As a consequence, poultry productivity is enhanced. Nutritional advances in feed additives include enzymes, synthetic amino acids, commercial preparations of vitamins/trace minerals, probiotics, prebiotics and toxin binders among others.

Feed enzymes are commercial enzyme products aimed at augmenting the endogenous enzymes secreted in the gut of birds when fed in the diet so as to improve availability of nutrients. Researches on enzyme usage is necessitated by wide use of vegetable proteins (e.g. legumes and oilseed meals) and some cereal grains in poultry diets.

The use of vegetable proteins was in response to a total ban on the use of animal protein sources in feeds by the European Union [31]; which hitherto was the main protein source in poultry diets due to their high digestibility. However, these vegetable proteins are characterised by high levels of anti-nutritional factors such as indigestible non-starch polysaccharides, phytic acid, tannins and alkaloids; which affect utilisation of nutrients.

Besides, some feed grains such as wheat, barley, sorghum and rye contain appreciable amounts of soluble non-starch polysaccharides that can cause very viscous gut content. Hence, the advances made in feed enzyme research culminated in the use of feed enzymes to depolymerise the soluble and insoluble pectic polysaccharides [32, 33] as well as hydrolyse phytates [34] in diets containing high levels of vegetable proteins and some cereal grains. In fact, feed enzymes prevent severe imbalances of nutrients that can remain undigested and reach the large intestine; thereby creating favourable environment for pathogens and consequent disease conditions. A good number of studies involving enzymes in poultry nutrition has been reviewed which have demonstrated improvements in feed utilisation with enzyme supplementation [35–37].

Additional beneficial effects of feed enzymes, particularly phytase, in cereal and oilseed- based diets include the following: release of digestible phosphorus to reduce the use of expensive supplemental inorganic phosphorus (e.g. dicalcium phosphate); release of minerals such as calcium, magnesium, zinc and potassium which are complexed with the phytate molecule; and prevent excessive excretion of phosphorus in manure into the environment [34].

The bird's gut microbial ecology has implications for feed digestion and disease control. This has to do with maintenance of natural balance of beneficial and pathogenic microorganism populations in the gut. Thus, the use of antibiotics previously as feed additive tended to cause imbalance between these two groups of microbes by eliminating largely the beneficial microbes as well as cause meat contamination and resistance problems in humans [38, 39]. Hence, the introduction of probiotics/prebiotics/synbiotics (combination of pro-prebiotic) as replacement for antibiotics.

Probiotics are a culture of live microorganisms. The probiotic product may contain microbes such as *Aspergillus oryzae, Lactobacillus acidophilus, L. bulgaricus, L. planetarium, Bifidobacterium bifidium, Streptococcus lactis and Saccharomyces cerevisiae*. Some of the commercially available probiotics for poultry are Bactocell®, BioPlus 2B®, Cylactin®, Lactobacillus acidophilus D2/CSL®, Microfern®, Oralin®, Protexin® and Thepax®. These products promote growth of beneficial microbes which competitively exclude the pathogenic microbes [40] and may as well secrete enzymes which aid digestion of feed.

The use of prebiotics in animal nutrition post- dated probiotics with favourable effects on poultry health and meat quality [41, 42]. It has been defined recently as "a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host' [43]. Commonly used prebiotics are mannanoligosaccharides (MOS), fructooligosaccharides and inulin. Generally, prebiotic products are resistant to attack by endogenous enzymes and therefore reach the site for proliferation of gut microorganisms, where they interfere with colonisation of the pathogenic microbes and thereby exclude them [44].

Inclusion of synthetic essential amino acids in poultry has been on the rise as a result of improving efficiency of protein utilisation. This is in line with current trend of formulating diets based on digestible amino acids in order to reduce amount of dietary protein [45]. These are DL-Methionine, L-lysine HCl, L-Threonine and L-Tryptophan. Initially, the idea was to meet first-limiting amino acids (Lysine and Methionine) requirements for poultry; however, with the need to further reduce dietary protein requirements as well as reduction in nitrogen excretion, this calls for increased use of second and third limiting amino acids such as L-Threonine and L-Tryptophan as well as the next limiting amino acids including Isoleucine, Valine and Arginine [45–48].

Advances in dietary supplementation with vitamins (water/fat soluble) and trace minerals (Zn, Cu, Mn, Se, Co, Fe) continue to be made and have brought about improvements in feed utilisation, growth performance and welfare of birds [49]. Vitamins and trace mineral supplements are vital feed additives aimed at supplying sufficient amounts that meets the needs of the biochemical systems of the cells of birds culminating in varied benefits. These benefits include enhanced mineral uptake, improved immune system, proper tissue and bone development, cellular growth as well as amelioration of oxidative stress in birds [49].

The use of toxin binders in poultry feeds has counteracted problems of mycotoxins associated with cereal grains [50, 51].

4.6 Use of nano-minerals

The use of nano-minerals in poultry nutrition is a recent concept that is gaining grounds as a result of varied application of nanotechnology in animal production systems. This has to do with alteration of particle size to few nano meters (1–100 nm) and studies reviewed so far have proved that feeding of nanoparticle improved digestive efficiency, immunity, growth rate, performance, resistance to pathogens, quality of meat and eggs in birds [52, 53].

4.7 Combating metal toxicity

Several researches have highlighted the problems of metal toxicity in poultry; which led to the establishment of nutritional guidelines on safety levels to protect both birds and humans from metal toxicity [54]. Nutritionally beneficial ones for good health but whose dietary excesses create health problems include iron, copper, manganese, zinc, etc.; whereas poisonous ones which may contaminate feeds from the environment include arsenic, mercury, lead, cadmium, vanadium, etc. [55, 56].

4.8 Production of designer eggs

Studies on dietary modification have played a major part in the production of nutritionally-improved eggs referred to as "Designer Eggs". By way of nutritional manipulations of the cholesterol content and its fractions, lipid profile, fatty acids, amino acids and minerals can be modified to produce healthy eggs for humans [57]. This can also be done through addition of therapeutic pharmaceutical compounds [57].

4.9 Nutritional management, gastro-intestinal tract conditioning and poultry health

It's undeniable fact that achievement of productive efficiency can only be attained through nutrition if and only if the health status of birds is not compromised. Therefore, current nutritional researches are geared towards optimising poultry health and consequently their welfare. Some of these nutritional strategies with positive impact on poultry welfare have been reviewed extensively [58]. They include: manipulation of diet composition (e.g. dietary ME/CP as a way of controlling the body composition to prevent body fatness of market broilers or fatty liver haemorrhagic syndrome in layers); addition of essential fatty acids such as linoleic acid and linolenic acid to prevent lesions or supplemental fats/oils to increase dietary ME values; use of calcium and phosphorus (an approximate ratio of 2: 1), or vitamin D to prevent bone problems such rickets and tibial dyschondroplasia or cartilage abnormalities that can lead to welfare problems such as osteomyelitis and femoral head necrosis; use of major supplemental mineral like sodium/vitamin and trace mineral supplements to boost normal health and/or under adverse conditions; use of feed additives such as enzymes and probiotics discussed above (Section 4.5) to improve feed efficiency with added advantage of less sticky excreta (better litter quality with less incidence of hock burns) and control of disease causing organisms, respectively; dietary modifications to help birds cope with stress, particularly under hot climatic conditions (e.g. decreasing crude protein content, use of synthetic amino acids to increase amino acid intake, use of fat to help decrease heat increment, use of sodium supplement as bicarbonate for maintenance of blood electrolyte balance, use of vitamins such as vitamins C, E and A to help in heat and other types of stress); and physical manipulation of feed such as mash feeding and feed restriction to control growth for maintenance of good health (e.g. lower mortality, reduction in metabolic disorders, improved walking ability).

5. Future nutritional research focus

Advances in poultry breeding, emergence of new feed resources, consumer demands and climate change as well as environmental concerns have implications for future poultry nutritional research. Thus nutritional research focus, henceforth, will be influenced mostly by the following innovations:

- Feed formulation softwares and feeding programmes
- Novel feed ingredients and feed additives
- Gastro-intestinal conditioners for gut health, birds' welfare and food safety
- Modern technologies for feed processing and feeding packages
- Perinatal nutrition and epigenetic programming

6. Future research funding

A balance should be maintained between a long-term and short-term research funding. Critical challenges needed to be addressed include the following:

- Scarce research funds, particularly in developing countries
- Prioritisation of limited research funds for nutritional research
- Basic research is relatively expensive and may hamper research in developing countries
- Diversion of research funds away from researches already conducted in developed countries that are relevant for application in developing countries
- The emergence of new demands from consumers, new disease situations, requirements to thrive on different feeding programmes and environmental conditions, have created new avenues for future research
- In-country competitive research funds (i.e. coordination of research at a national level to avoid multiplicity of similar research)
- Animal welfare (e.g. hot climate, dietary manipulation, growth rate, etc.)
- Food safety [e.g. mineral residues, pathogens (*Salmonella*, *E. coli*, *Campylobacter*, etc.)]
- Limitation (in use of antibiotics)
- Disease risks
- Competition between poultry and humans for feed ingredients (e.g. maize)

7. Conclusions

Nutritional research has contributed significantly to poultry production over the years. Its role in the poultry sector is more crucial than ever before in sustaining progress made in the sector as world population continues to increase at alarming rate. The progress in nutritional research is made possible by several advanced techniques that have been developed and tested by numerous researchers both in academia and industry. The appropriateness of any techniques to be used depends on the facilities available at the research site and the cost involved. Also, there is a need to maintain balance between research that may have future usefulness (fundamental research) and that which may be used immediately (applied or practical research) through application of scientific innovations. In future, nutrition objectives will require scientists to use extensive interdisciplinary approaches.

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Chapter 2

Nontraditional Feedstuffs as an Alternative in Poultry Feed

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Abstract

Soybean meal and yellow corn are conventional feedstuffs used as the main ingredients in poultry feeds due to their high nutrients availability. On the other hand, these two feedstuffs are high in demand by other animals (soybean meal) and humans (yellow corn). By the year 2050, the world's population is expected to increase up to 9.1 billion. Global consumption of poultry products, such as meat or eggs is increasing predominantly in developing countries. Consequently, the global demand for poultry feedstuffs would increase. The availability of feedstuffs for poultry nutrition nowadays is becoming more competitive. Thus, food security, especially in the developing countries, would be threatened. Currently, efforts are being made to use alternative feedstuffs to substitute portion of soybean meal and yellow corn in poultry diets. This chapter discusses the alternative feedstuffs that can be incorporated in poultry feeds. In addition, the nutritive content and availability are examined as well as how to improve the nutritive quality of such nontraditional feedstuffs.

Keywords: alternative feedstuffs, insects, algae, poultry feed

1. Introduction

Due to their high nutrients contents, soybean meal and yellow corn are conventional feedstuffs in poultry feeds. Moreover, these two feed ingredients are also high in demand by other animals (soybean meal) and humans (yellow corn). The global consumption of poultry products, such as meat or eggs, appears to be increasing in the developing countries. Therefore, the global demands of the main poultry feedstuffs would increase leading to higher cost of poultry production. Studies have shown that the world's population is expected to increase to 9.1 billion by the year 2050, [1]. This tremendous increase in population would produce competition in the available poultry feed ingredients for poultry nutrition. Furthermore, this increase in population will increase demand for poultry products. As a result, the availability of feed ingredients for poultry nutrition would become more competitive. In addition, there is an increasing trend to produce biofuel from feedstuffs, especially corn, to meet the demand all over the world. This further poses a serious food security risk, especially in the developing countries.

Currently, efforts are being made worldwide to use alternative sources of protein and energy to be substituted for soybean meal and yellow corn in monogastric animals such as poultry and swine. It is known that some developing countries produce a huge amount of alternative feedstuffs, considered as agro waste by–products such as wheat bran, rice bran, cotton seed meal, copra meal and palm kernel cake. However, many of these agro waste by–products are featuring on presence of non-starch polysaccharides (NSPs) such as xylan and mannan as well as anti-nutritional factors [2].

The NSPs are found to be the main reason for increasing the viscosity in the small intestine of the birds, and hence lead to increased moisture content of the excreta. Hence, the productivity and health status of the chickens could be affected [2]. Therefore, the inclusion of these agro waste by–products in poultry feed are limited. The nontraditional feedstuffs can be defined as those feed ingredients that have not been conventionally or commercially used in poultry rations. This chapter discusses the nontraditional feedstuffs with potential to be replaced partially or totally with soybean meal and yellow corn in poultry feeds.

2. Alternative feed ingredients for yellow corn

It is well known that yellow corn is used as a main source of energy ingredient in poultry diets [2]. There are some nonconventional feed ingredients that can substitute certain amount of yellow corn in poultry rations. However, there are some limitations such as presence of anti-nutritional factors that lead to decrease feed intake and growth performance (**Table 1**). The other important point to consider is that the lack of knowledge about the composition of nutrients and their availability, due to the lack of research centers in the developing countries limit use of these feed ingredients.

2.1 Sorghum

Sorghum is the main food grain in Africa and parts of India and China [3]. The nutritive value of sorghum is almost 90–95% similar to that of yellow corn. Moreover, its global price is less than yellow corn [4]. The problem of sorghum is the high tannins content, which is water soluble polyphenolic metabolites and leads to reduce growth performance of poultry. Tannins in higher concentration are anti-nutritional because made chelates and reduce protein digestibility [5]. Sorghum is usually classified as bird resistant (less than 0.5% tannin) or non-bird resistant (1.5% tannin or higher) varieties. The negative effects of tannins are decreasing growth, feed intake, protein digestibility, egg production and leg abnormalities with broilers [4]. There are some procedures that can be applied to the sorghum to minimize tannins and improve the nutritive value of such feed ingredients. These methods include soaking in alkali solution and water. It is reported that tannic acid can be hydrolyzed in the chicks to gallic acid which excreted in urine as

Ingredient	Limitation
Sorghum	High tannins content.
Wheat bran	High fiber content, low metabolizable energy.
Distillers dried grains with solubles (DDGS)	Variability and availability of nutrients.
Date wastes	High fiber content in the date pits, low lysine, methionine, leucine and isoleucine.
Millets	High fiber and tannins.

Table 1.
Alternative energy sources that can replace yellow corn in poultry diets.

4 – O – methyl gallate [4]. Therefore, the action of methyl donors such as calcium hydroxide or slurry of sodium carbonate could be included in poultry rations to improve the feed intake of high tannin sorghum. As a result, low tannin sorghum can completely replace yellow corn in poultry diets.

2.2 Wheat bran

Wheat bran is the outer seed coat from flour mills. High in fiber, low in metabolizable energy (ME) and its usage in poultry nutrition is limited [4]. The ME can be increased up to 10% by simple steam pelleting, and the availability of phosphorus up to 20% under the same condition [6]. This by product could be beneficial for gut health which is reported to modify the gut microflora [4]. It is reported that wheat bran can be added in poultry diets up to 5–8% without negative effect [4]. Wheat bran contains xylan which may lead to increase viscosity in the small intestines. Therefore, xylanase supplementation is recommended for broilers fed more than 15% wheat bran in their diets [4].

2.3 Distillers dried grains with solubles (DDGS)

Alternative and clean sources of energy are more attractive nowadays against fossil energy. The production of biofuel has globally increasing [7]. Therefore, the by-product obtained from this process is known as distillers dried grain with solubles (DDGS). It can be defined as a product obtained after ethanol extraction by distillation from the yeast fermentation, and drying at 75% of the resultant [8]. Including DDGS in poultry diets to replace part of yellow corn and soybean meal have shown positive results in terms of growth performance [9]. The main limitation of using DDGS in monogastrics is the variability of its nutrients content and availability [9]. This is due to the variation of growing conditions, ethanol production method and oil extraction. Therefore, it was reported that there are two types of DDGS; high protein and conventional DDGS (**Table 2**).

Nutrient	High protein DDGS	Conventional DDGS
Dry matter	83.10	89.80
Crude protein	34.10	27.10
Crude fiber	8.35	7.85
Ether extract	7.91	9.63
Arginine	1.49	1.10
Cystine	0.58	0.45
Glycine	1.25	0.60
Histidine	0.88	0.62
Isoleucine	1.26	1.15
Leucine	4.32	2.40
Lysine	1.16	0.70
Methionine	0.74	0.50
Phenyl alanine	1.57	1.35
Serine	1.60	1.30
Threonine	1.31	0.93
Tryptophan	0.30	0.20

Nutrient	High protein DDGS	Conventional DDGS
Tyrosine	1.34	0.80
Valine	1.60	1.40
Metabolizable energy (Kcal/Kg)	2628	2628

Table 2.
Nutrient composition of DDGS (% as –fed basis) [9].

Not only can DDGS provide energy in poultry diets, but also can provide protein and available phosphorus. It was shown that DDGS can be included in broiler diets at 8% or 15% in starter and grower phase, respectively without negative effects in their performance [8]. The supplementation of fiber-degrading enzyme could be an efficient way to enable the use of increased levels of DDGS in poultry and pig diets [10].

2.4 Date wastes

Dates are rich in vitamins and minerals. Usually, dates wastes consisting on the pulp and pits (stones). Date wastes are high in fiber, low in lysine, methionine, leucine and isoleusine [11]. The limitation of using date wastes is the high crude fiber in the date pits. Date wastes can be included in poultry diets up to 30% without negative effects on their performance [12]. In addition, the use of 30% of date pits (stones) with a supplementation of multi enzymes in broiler diets had no adverse effects on the final body weight [13]. Regarding date pits meal, it could be fed to laying hens up to 5% without adverse effects on their performance and egg quality. In addition, broilers fed diet incorporated with 4% date pits meal showed an ability to resist the deleterious effects of aflatoxine B1 [14].

2.5 Millets

Millets is adrought-resistant plant that produces a nutritious grain. It can be grown successfully under environmental conditions where corn and wheat fail to survive [15]. The nutrient content is variable, so that it contains 8–10% CP, 3395–3738 kcal/kg metabolizable energy, 3.60–5.27% fat and 1.59–2.36% fiber [15]. The limitation of using high levels of millets in poultry diets is the tannin content and fiber [16].

3. Alternative feedstuffs for soybean meal

Routinely, soybean meal is used as a main source of protein ingredient in poultry diets [4]. There are some nontraditional feed ingredients that can replace certain amount of soybean meal in poultry diets. Nevertheless, there are some limitations such as presence of anti-nutritional factors that lead to reduce feed intake and growth performance (**Table 3**).

3.1 Canola meal

Canola crop is growing widely in the west of Canada as well as in other parts of the world [4]. The production of canola was influenced by the increasing demand for canola oil. Canola meal is the by-product of oil extraction, and lysine content is less than that of soybean meal. However, sulfur-containing amino acids are higher than that of soybean meal.

Ingredient	Limitation
Canola meal	Presence of glucosinolates, senapine, phytate, fibers, tannins, and low metabolizable energy.
Peanut (groundnut) meal	Trypsin inhibitors, potential aflatoxin contamination.
Peas	Lack of sulfur containing amino acids, and moderate energy levels
Lupins	High fiber, low metabolizable energy.
Sesame meal	High levels of phytate.
Blood meal	Palatability and low growth rate.
Palm kernel meal	High fiber, coarse texture and high NSPs.
Cottonseed meal	High fiber, gossypol, dry and dusty nature, phytate, sterculic acid.
Feather meal	Low in amino acids availability.
Insects and worms	Microbial deterioration and lipid oxidation during storage.
Earthworms	High fat (PUFA), and lipid oxidation during storage.
Algae	High fat (PUFA), and lipid oxidation during storage.
Azolla	High fiber content.
Single – cell protein	High fat (PUFA), and lipid oxidation during storage.

Table 3.
Alternative protein sources that can replace soybean meal in poultry diets.

The problem of using canola meal in poultry feeds is the presence of glucosinolates, senapine, phytate, fibers, tannins as well as it has low metabolizable energy [17]. It was found that feeding canola meal to layers led to the occurring of fishy taint in egg and the reduction egg size [4].

There are attempts to improve the nutritional quality of canola meal by extrusion or solid-state fermentation using lactic acid bacteria [6, 18]. Therefore, it was reported that canola meal can be incorporated in poultry diets up to 5–8% [4], or up to 10% in broilers fed fermented canola meal based diet [17].

3.2 Peanut (groundnut) meal

Peanut meal is a by-product from oil extraction. It contains 0.5–1% oil and 47% CP. The problem of using peanut meal in poultry diets is the trypsin inhibitors. Fortunately, it can be detoxifying by heat treatment during oil extraction. The issue to consider is that its potential aflatoxin contamination. To overcome this problem, the feedstuff could be supplemented with sodium-calcium aluminosilicates because these minerals bind with aflatoxin preventing its absorption [4].

3.3 Peas

Peas can be used in poultry diets depending on local economic conditions. It contains moderate amount of energy and protein. The limitation to use peas in poultry rations is the lack of sulfur containing amino acids, and moderate energy levels [4].

3.4 Lupins

The use of low alkaloid lupins in poultry diets is going to be increased in certain regions of the world [4]. The high level of fiber in the seed leads in low metabolizable energy compared to soybean meal. Although lupins are much lower in methionine and lysine, many reports suggested that sweet lupins are comparable to soybean meal in terms of protein quality [4].

3.5 Sesame meal

Sesame meal is very deficient in available lysine. It contains high level of phytate which may cause problems with calcium absorption. Therefore, skeletal disorders or poor egg shell quality in laying hens may be occurred. It contains 35.1–47% CP [16]. It is recommended that diet incorporated with more than 10% sesame meal should be increased by 0.2% extra calcium [4].

3.6 Blood meal

Blood meal is high in protein (65–85%), rich in lysine, arginine, methionine, cysteine and leucine. However, it is very poor in isoleucine [19]. The use of blood meal is very limited in poultry diets because of its palatability and poor growth rate [4]. It was reported that blood meal can be incorporated up to 3% in broiler diets without negative effects in their performance [19].

3.7 Palm kernel cake

Tropical regions have an abundant amount of palm kernel cake (PKC), which is considered an agro-industrial waste derived from the extraction process of oil from palm fruits. It has been used in poultry diets as an alternative to soybean meal. Nevertheless, the use of PKC is limited in monogastrics because of its high content of fibers, coarse texture, and non-starch polysaccharides (NSPs) [2, 20–24]. The main NSPs in the PKC are mannan, xylan, arabinoxylan, and glucoronoxylan [20]. This is considered a significant issue faced by nutritionists, and it has limited the use of PKC for manipulation of feed formulation. It has been reported that 10% is the maximum level of PKC that can be given to broiler chickens. However, solid-state fermentation by cellulolytic bacteria may improve the nutritive value of PKC to be incorporated up to 15% in the diet [2, 24].

The treated PKC by enzyme [25], cellulolytic bacteria via solid state fermentation [2, 23, 24] or extrusion [26] may contribute to improve the nutritive value and poultry performance (**Table 4**). It was reported that extrusion led to 6% increase in apparent metabolizable energy and 32% in crude protein digestibility in broiler chickens [27].

3.8 Cottonseed meal

Cottonseed meal is a byproduct after oil extraction. Usually, this byproduct used for poultry in cottonseed producing regions [4]. It is high in crude protein (41%). However, the big problem for using cottonseed meal in poultry rations are the high fiber levels (14.5%) and gossypol [4]. Gossypol is a yellow polyphenolic pigment, and usually found at 0.1% free gossypol. The big issue with gossypol is binding with lysine during processing, and then the lysine will be unavailable to the chickens. The byproduct is not acceptable by poultry because of its dry and dusty nature [3]. Gossypol may lead to decrease feed intake and growth rate in broiler chickens [3]. The byproduct is low in calcium, and the phosphorus is chelated with phytate. Therefore, phytase supplementation could be beneficial to release unavailable phosphorus. In case cottonseed meal is used for poultry, it is recommended to supply fish meal to balance the essential amino acids and calcium [3].

Nutrient (%)	PKC [23]	FPKCa[1] [23]	FPKCb[2] [23]	PKC [26]	Extruded PKC [26]
Crude protein	16.43	16.80	16.68	16.90	16.90
Dry matter	91.42	92.62	92.44	89.81	91.79
Ash	474	4.67	4.80	4.50	5.70
Crude fiber	16.96	14.09	14.29	17.30	14.60
NDF	82.29	71.70	73.54	75.00	75.40
ADF	51.48	47.27	47.45	37.30	39.30
Indispensable amino acids					
Lysine	0.37	0.41	0.38	0.5	0.46
Leucine	0.89	0.94	0.95	1.08	1.05
Isoleucine	0.50	0.59	0.53	0.60	0.55
Valine	0.69	0.78	0.72	0.90	0.87
Phenyl alanine	0.57	0.66	0.63	0.66	0.57
Threonine	0.41	0.51	0.46	0.54	0.50
Histidine	0.23	0.29	0.24	0.31	0.31
Methionine	0.22	0.27	0.26	0.30	0.28
Arginine	1.60	1.76	1.69	1.94	1.95
Glycine	0.60	0.78	0.71	0.80	0.81
Dispensable amino acids					
Aspartic acid	1.12	1.27	1.23	1.14	1.15
Glutamic acid	2.48	2.80	2.76	3.06	3.17
Proline	0.44	0.59	0.52	0.57	0.53
Serine	0.56	0.69	0.66	0.75	0.74
Tyrosine	0.25	0.24	0.24	0.30	0.31
Cysteine	0.20	0.22	0.21	0.36	0.17
Alanine	0.62	0.70	0.71	0.87	1.10

[1]*FPKCa; fermented palm kernel cake by P. polymyxa ATCC 842.*
[2]*FPKCb; fermented palm kernel cake by P. curdlanolyticus DSMZ 10248.*

Table 4.
Nutrient content of palm kernel cake and treated palm kernel cake (dry matter basis).

The other important point to consider with gossypol is that it leads to discoloration of the yolk in laying hens. It causes a olive-green color in the yolk, especially during egg storage at low temperature [3, 4]. The other problem with cottonseed meal is the presence of sterculic acid witch found to cause a pink color in the albumen. However, this can be avoided by using a byproduct with less residual oil because of the content of cyclopropenoid fatty acids [5].

It has been found that iron can bind with gossypol by 1:1 ratio, and may detoxify the gossypol. Therefore, the addition of 0.5 kg ferrous sulfate/tonne allowed the broilers and layers to tolerate up to 200 ppm and 30 ppm free gossypol, respectively without any negative effect in their performance [4].

In case iron was supplemented to cottonseed meal based diet, the balance between iron and copper should be considered to be 10: 1 iron to copper, respectively.

Studies have also shown that enzyme supplementation (β-glucanase and xylanase) may lead to increase the metabolizable energy and protein utilization in broiler chickens [28].

3.9 Feather meal

Feathers are considering as an industrial waste resulted during birds processing in slaughter houses. Several million tons of feathers are generated from the poultry processing industry are disposed as a waste [29, 30]. Feather meal contains about 85% crude protein, 5% cysteine and 3000 kcal/kg metabolizable energy. The cysteine availability is about 60% depending on the processing conditions [4].

Usually, feathers are partially dried, and hence steam-treated to introduce hydrolysis. However, the extreme temperature will lead to destruct the amino acids, especially lysine. Therefore, leads to reduce the amino acids digestibility. To overcome this problem, the use of keratinase enzyme may play an important role in improving the protein digestibility [29] and poultry performance [4]. In addition, fermentation with bacteria-degrading keratin such as *Bacillus licheniformis* for five days at 50°C can produces a fermented product comparable in nutritional value to soybean meal [4].

Some reports mentioned that *B. subtilis* and *Aspergillus fumigatus* had an ability to degrade keratin in feathers [30]. Feather meal can be included in poultry diets at 2–3%. Nevertheless, the fermented feather meal may give promised results in poultry nutrition, and therefore it would be an additional commercial benefit for the poultry industry by replacing part of soybean meal in poultry feeds.

3.10 Insects and worms

Insects can be used to produce cheap source of protein. It is known that insects are considered as a natural food for birds. Insects are rich in protein (40–76%) and essential amino acids [31], particularly sulfur containing amino acids [32]. Insects meal are usually featuring on high fat content [31]. Therefore, microbial deterioration and lipid oxidation should be considered during storage [33]. Ssepuuya et al. [34] indicated that insects meal may replace the conventional protein sources by 10–100% without any negative growth performance whether in fish or poultry. It was also mentioned by Kareem et al. [31] that the incorporation of black soldier fly larvae to broiler diets up to 10% had no negative effect in their growth performance under humid tropical environment. In addition, no adverse effects on growth performance, carcass characteristics, hematological and serum biochemical indices in growing Japanese quail when meat and bone meal replaced with *Spodoptera littorails* in their diets [35]. It was claimed by Neumann et al. [36] that partly adding defatted insects meal of *Hermetia illucens* larvae in broiler diets – 26% and 22% in starter and grower phase, respectively – were acceptable. In terms of meat quality, it was reported that complete substitution of soybean meal by *Hermetia illucens* led to inducing lipid oxidation in broiler meat [37]. This was attributed to the high content of poly unsaturated fatty acids (PUFA) in *Hermetia illucens*.

3.11 Earthworms

Earthworms are a natural source of protein for poultry raised in free-range system. Earthworm can produced even in small-scale system. Earthworms species require a temperature ranging from 15 to 25°C, and 60–85% soil moisture content [38]. It can be considered as an alternative source of protein (64–76%) [39]. At the same time, it can be degrade animal manure to clean the environment. It was reported that the total essential amino acids in earthworms are comparable with egg protein. Moreover, the omega – 3 PUFA are quite high and similar to that of some fish oil [40]. It was mentioned by Parolini et al. [38] that earthworms contain

6–11% fat, 5–21% carbohydrate, 2–3% minerals and range of vitamins, especially niacin and cyanocobalamin. In comparison with insects meal, it has been found that earthworm meal has no deficiencies in the essential amino acids and better fatty acids profile with no chitin content, so that it was more acceptable and palatable for chickens [38]. Earthworm meal could be integrated in broiler diets up to 10% without negative effects in growth performance and meat quality [38].

3.12 Algae

Algae represent an important source of unconventional protein (50–60%), oils, vitamins, minerals, antioxidant and colorants [41], carotenoids, omega-3 and omega-6 PUFA [42, 43]. Some types of algae contain up to 76% crude protein [44]. In terms of nutrition, algae were used in broiler diets up to 16% without adverse effects. On the other hand, it was a replacement for approximately 60% of soybean meal and 40% of animal vegetable blended fat into practical broiler diets [44].

The most common species of algae used in poultry nutrition are *chlorella* and *Spirulina*. It was reported by Moury et al. [45] that supplementation of *Spirulina platensis* in broiler diets may completely replace the incorporation of vitamin-mineral premix. Moreover, it can be substitute the antibiotic usage in animals [46].

It is reported that algae can be a good option for 100% organic poultry feed [47]. Neumann et al. [36] reported that incorporation of *Spirulina platensis* at 21% and 17% in starter and grower phase, respectively was acceptable. However, nutritionists have to pay attention to the presence of PUFA in algae which may affect the meat quality of broilers and lead to lipid oxidation. Gkarane et al. [37] mentioned that complete substitution of soybean meal in broiler diets by *Arthrospira platensis* influenced the meat quality and led to lipid oxidation.

3.13 Azolla

Azolla is an aquatic and floating fern of the family Azollaceae. It contains 25–35% crude protein, 10–15% minerals and 7–10% amino acids, especially lysine [48]. Azolla forms a symbiotic with blue green algae which lives within its leaves. This relationship makes azolla as a beneficial source of protein, and can be fed safely to the farm animals [49]. It is recommended that azolla (*Azolla pinnata*) can be incorporated in poultry diet up to 5% with positive effect on their growth performance [49]. The limitation of using high levels of Azolla is its high level content [48].

3.14 Single-cell protein

The production of single-cell protein (SCP) can be done by microbial fermentation with selected strains of microorganisms. SCP also known as microbial protein or bio-protein [50]. Bacteria such as *Pseudomonas* spp. can be grown in methanol, ethanol and organic acids [3]. The protein and sulfur containing amino acids in bacteria are higher than that of yeast. The oil content in bacteria and yeast is high and rich in unsaturated fatty acids. Chen et al. [51] concluded that SCP produced by *Chlostridium autoethanogenum* had 88.93% crude protein and most of essential amino acids were higher than that of fish meal.

The incorporation of 15% of SCP in pigs diet exhibited a comparable results with those group of pigs fed diet containing soybean meal [3]. It is recommended that SCP can be included in 2–5% in broiler diets, and up to 10% in laying hens [3].

4. Alternative ingredients for oil and vitamins sources

It is known that – ingredients mentioned above – insects, worms, earthworms, algae, azolla and SCP contain significant amount of oil. In addition, these ingredients can provide omega-3 and omega-6 PUFA to the poultry [42, 43]. Interestingly, these ingredients are rich in vitamins and minerals as mentioned above [46].

5. Conclusion

In conclusion, the use of alternative feedstuffs nowadays in poultry sector is going to be increased because of their nutritive quality and as a cheap source of protein and energy. In addition, these nontraditional feedstuffs are not competitive with humans. At the same time, their inclusion to poultry diets can replace portions of soybean meal and yellow corn. Therefore, reduce the cost of production.

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Chapter 3

Microbial Hydrolysed Feather Protein as a Source of Amino Acids and Protein in the Diets of Animals Including Poultry

Jitendra Kumar

Abstract

Feathers are hard waste products, mainly composed of hard β-keratin, and are produced in large quantities in commercial poultry processing plants. Therefore, their industrial utilization is important economically as well as environmentally. Feathers degradation through keratinolytic microorganisms has been considered as an important method for efficient bioconversion, nutritional enhancement and eco-friendliness. The use of crude keratinase significantly increased the amino acid digestibility of raw feathers and commercial feather meal. This enzyme increased the digestibility of commercial feather meal and could replace as much as 7% of the dietary protein for growing chicks. However, feathers are currently utilized on a limited basis as a dietary protein supplement for animal feed because feather meal production is an expensive process, requiring significant amounts of energy. This review paper explains the nutritive value of feathers which makes suitable and inexpensive animal and poultry feed.

Keywords: feather, feather meal, keratin, animal feed, keratinase, protein hydrolysate

1. Introduction

Rising livestock is a major industry, which produces animals that have multiple uses as meat, fibers and hides. It is important to feed the stock animals a proper balanced diet, to insure optimum growth and health. Feed Industries are seeking new way to cope with raw materials costs [1]. Viable treated feather and hog hair meals have been found valuable sources of dietary protein for the growing chick [2]. Recently, supplementation of poultry diets with enzyme mixtures, including protease and amylase has produced improvements in growth performance [3]. Keratinase is an enzyme hydrolyses a broad range of protein substrate including casein, collagen, elastin and keratin [4]. Scientist reported degradation of chicken feathers and other keratinous waste materials by fungi [5] and decomposed feathers were utilized as nitrogenous fertilizers due to their high value protein content [6, 7]. This feature of keratin protein can accomplish the shortage of meat raw materials and achieve desire of manufacturer to reduce production costs, and the availability of alternative sources of protein [8]. Feather degradation by microbial action seems to be a reasonable substitute to obtain feather meal that would be nutritionally raised with essential amino-acids. This line of biodegradation of chicken feathers would convert the rigid feather waste to a readily digestible feather meal.

2. Keratin

Keratin is hardened fiber plus matrix material which ultimately fills the cells of hair cortex. It thus consists of two main components; a fibrous protein which gives the α-keratin x-ray diffraction pattern (or the β-pattern when the polypeptide chains are extended as in feather keratin) and an amorphous protein which is termed γ-keratin [9]. Only rare microorganisms like fungi, bacteria and actinomycetes are capable to break and utilize keratin because of their hard and tough nature. Humans and other vertebrates cannot digest this macromolecule, and if eaten, it simply gets accumulated within the variety of a lump that is still undigested. A large part of tiger scat and other carnivore dung contains scleroprotein (mainly hair) aside from bones and additional complex elements which are undigestible [10]. Animal hair, hoofs, horns and wool contain β-keratin and bird's feather contains α-keratin. Keratins are also present in epithelial covering which is rich in beta helical coil linked through cysteine bridges [11]. The higher the percentage of sulfur, the higher is the stability of keratin towards solubilization [12]. The keratin proteins are compound that are extremely resistant to action of physical, chemical and biological agents. Hair, horns, nails and cornified tissue are some naturally occurring forms of keratin [13–14]. Keratin is a protein macromolecule with very high stability and low degradation rate.

Keratins are categorized into hard and soft keratins according to the sulfur content. Hard keratins have high content di-sulphide linking and are found in appendages. Soft keratins have low content of disulphide bond making skin and callus [15]. Keratins belong to the super family of IF protein. Intermediate filament proteins are planned, prolonged α-helical conformation prone to form two-stranded coiled coils. The durability of keratins is a direct consequence of their complex architecture [16].

2.1 Chicken feathers

The main component of feather is keratin, a mechanically durable and chemically unreactive and insoluble protein, which render it difficult to be digested by most proteolytic enzymes. Keratin is resistant to enzymatic digestion by plant, animals and many known microbial proteases due to insoluble nature. Feathers having only 10% parts which is not keratin, rest 90% is resistant to degradation by common peptidases. This resistance is due to constituent amino acid composition and configuration that provide structural rigidity [17]. Chicken feathers are made up of over 90% of keratin protein, small amounts of lipids and water. Feathers contain about 15% N on a dry weight basis and huge quantities are produced as industrial by product. However, they have not been used effectively as plant bio fertilizers since N mineralization are slow meet plant requirements [18]. Feather waste, resulting in large quantities as by product of poultry farms processing, are pure keratin proteins.

3. Keratinases from microorganism

The keratinase producing micro-organisms have been discovered in several different biological groups, including fungi, bacteria and actinomycetes.

3.1 Bacterial strains

The genera *Burkholderia*, *Chryseobacterium, Pseudomonas* and *Microbacterium* sp. were grown on solid medium with feather meal as sole carbon and chemical elements and screened for proteolytic activity on milk agar plates [19]. Three *Bacillus* species were isolated from the poultry industry and evaluated for keratinase production using feathers or feather meal as the sole carbon and nitrogen sources in a submerged fermentation. *B. subtilis* 1273 was the strain which exhibited the highest enzymatic activity [17]. A number of keratinases producing *Bacillus* and *Pseudomonas* species have been isolated from various environmental sources such as soil farm wastes and raw feather [20]. *Bacillus* sp., *Bacillus licheniformis*, *Bacillus subtilis* KD-N2, *Burkholderia* was isolated for keratinase production [21–22].

3.2 Actinomycetes strains

Thiosulfate production from cystine by keratinolytic prokaryote *Streptomyces fradiae* [23]. Biochemical mechanism of keratin degradation by the actinomycete *Streptomyces fradiae* and the fungus *Microsporum gypseum*: a comparison [24]. Keratinolytic serine protease was purified and characterized from *Streptomyces albidoflavus* [25]. Native keratin decomposition by thermophilic *Actinomycetes* was studied [26]. Keratinase enzyme was isolated and characterized, which was produced during wool degradation process by *Thermoactinomycetes candidus* [27]. Thermoactinomycetes degraded keratin and other collagenous waste by alkaline hydrolysis [28]. A new strain of *streptomyces* was used to degrade feather [29]. A new actinomycetes was isolated from coastal region of south India and studied keratinase production [30].

3.3 Fungal strains

The thermophiles may be advantageous in comparison with mesophiles, because of their accelerated reaction processes and the accumulation of biomass and enzymes and diminished the risk of contamination in industrial activity. A large number of keratinases producing fungi were observed by [31]. 234 fungal strains were isolated by baiting method used for feather degradation and keratinase producing ability. Maximum clearing zone was made by *Chrysosporium indicum* on solid agar plates. The highest keratinase production was found in case of *Acremonium strictum* while *Chrysosporium indicum* and *Chrysosporium tropicum* was found next to it [32]. Fungal keratinase reported from India are listed in **Table 1**.

Fungal species	Aim of study	Author	References
Acrodontium album, Aspergillus ustus, A. quercinus, otryotrichum keratinophilum, Chaetomium globosum Chrysosporiumcrassitunicatum, C. tropicum, Curvularia indica, Gliocladium agrawalii, G. roseum,	Keratinase activity	Kushwaha	[33]
Geotrichum candidum	Keratinase	Rajak et al.	[34]
Trichophyton simii	Characterization	Singh	[35]
C. tropicum, C. keratinophilum, C. pannorum, C. queenslandicum, Malbranchea flava,	Keratinase	Kaul and Sumbali	[36]
C. keratinophilum, C. queenslandicum, C. tropicum, C. indicum, Malbranchea chrysosporoidea, Scopulariopsis brevicaulis, Curvularia lunata	Keratinase production	Kaul and Sumbali	[37]
Scopulariopsis brevicaulis	Dehairing process	Anbu et al.	[38]
Microsporum gypseum	Secretion of keratinase	Raju et al.	[39]
Acremonium strictum	Optimization of media composition	Kumar and Kushwaha	[40]
Cunninghamella echinulata	Purification & Characterization	More et al.	[41]
Aspergillus fumigatus	Purification of keratinase	Paul et al.	[42]
Acremonium strictum, C indicum, C. tropicum, C queenslandicum, C. pannicola,	Screening for keratinase	Kumar and Kushwaha	[32]
Aspergillus flavus	Optimization in SSF Condition	Mini et al.	[43]
Scopulariopsis brevicaulis	Statistical optimization	Satyalakshmi et al.	[44]

Table 1.
Fungal species producting keratinse.

4. Feathers as source of proteins and amino acids

An appealing alternating method to obtain amino acids and proteins is to use feathers that are relatively stable under natural conditions. Keratinophilic fungi used to hydrolyse keratin protein to obtain protein [33] and found maximum producer as *C. indicum*, *C. tropicum*, and *Malbranchea pulchella*. Parihar and Kushwaha [45] used *Verticillium tenuipes, Microsporum gypseum, Aphanoasus fulvescens*, *Chrysosporium keratinophilum* for study of protein release in hen feather degradation without rachis. *C. indicum* was used for degradation of human hair and estimated protein release 47.66 μg/ml and 112.66 μg/ml in 5 and 10 days respectively [46]. *Chrysosporium tropicum*, *Penicillium griseofulvum* and *Aphanoascus terreus* was analyzed for release of considerable amount of protein [47]. [48] observed protein release 409.6 μg/ml in case of *C. tropicum* in 12 days while *Malbranchea* sp. released 298.21 μg/ml in 4 days. [46] recorded 238 μg/ml in 25 days by *Alternaria tenuissima*.

Conversion of feathers into feather meals by applying physical and chemical methods results in the loss of nutritionally essential amino acids such as methionine, lysine and tryptophan. Therefore, currently the poultry feathers are converted into feather meal, a digestible dietary protein, for animal feed using keratinases. The microbial production of L-lysine is an expanding branch of manufacturing biotechnology. There are many reports are available worldwide. Indian researcher [37] studied the discharge of cysteine in the culture medium.

[47] recorded cysteine produced by *Acremonium strictum* 32.00 μg/mL, *Chrysosporium tropicum* 25.00 μg/mL, *Chrysosporium indicum* 22.00 μg/mL, *Malbranchea aurantiaca* 21.00 μg/mL. [48] Studied the discharge of amino acids lysine, cysteine, methionine, valine by *C. tropicum* and *Malbranchea* sp. due to feather degradation. [6] Found *A. tenuissima* a potent feather degrading fungus and increased the nourishing value of the soil by adding proteins (238 μg/ml), cysteine (20.2 μg/ml), lysine (15.8 μg/ml), methionine (6.8 μg/ml) and valine (7.5 μg/ml) in 25 days.

4.1 Animal feed

The upgradation of feather meal through microbial or catalyst treatment has been defined earlier. Feather meal fermented with *Streptomyces fradiae* and supplemented with essential amino acid methionine bring about in the broilers growth rate comparable with those fed isolated soybean protein [49]. The application of feather-lysate from *B. licheniformis* with amino acid supplementation formed alike development rate in chickens when compared to chickens fed with a diet included with soybean meal [50]. Feather hydrolysates produced by microbial keratinases have been used as additives for animal feed [51]. The application of biotechnological approach using microbes for feather processing has nutritional significance. Culturing of the microorganisms and keratinase activity may result in modification of structure of feather keratin [4, 52–54]. This may alter its resistance to digestive enzymes of the consuming animals [55]. Fermentation of feathers involving microorganisms and microbial enzymes, not only it would retain the existing valuable amino acid content of keratin, but it would also add to it. Thus, the feather meal obtained after such microbial treatment would have enough nutritional value. Keratinase could play a significant role in enzymatic improvement of feather meal and amino acid production from high molecular weight substrate [56]. The microbial technology would significantly bring down the cost since it would not require hydrothermal treatment, however feather waste would be a cheap raw material [57].

4.2 Feather meal as Chick feed

Meat and feather meal protein gave equally as good results as soybean meal protein when supplied 3% protein in practical-type corn-soybean meal rations [58]. [59] observed growth of feather and composition in broiler chicken and found that that of threonine, isoleucine and valine increased with age while methionine content of feathers decreased with age. *B. licheniformis* produced crude keratinase enzyme augmented the total amino acid digestibility of raw feathers and commercial feather meal, could replace as much as 7% of the dietary protein for growing chicks [19]. [60] Studied dietary crude protein and lysine amino acid effect on growth of feather in chicks and found that crude protein has more influence on feather development than by levels of lysine. [61] Observed antioxidant potential property of protein hydrolysate developed by *Bacillus* sp. [62] observed replacement of fish meal with feather meal in broiler and found economic without any negative effect. Treated chicken feather meal used as a source of protein ton animal feed broiler chickens [63]. [64] Observed processed feather meal for their chemical composition and amino acid profile and found feather meal pre-soaked with wood ash for twenty-four hour boiled at 150°C for 1 hr. gave the best crude protein content. Using feather waste as a valuable resource can help the poultry industry to dispose of the waste feathers in an environmentally sustainable manner that also generates extra income for the industry [65]. [66] Improved digestibility of protein into feather meal by enzymatic treatment. Feather meal can be included in the broilers diet without any negative effect on its performance [67]. [68] Studied effect of hydrolysed feather meal on feed efficiency, survival rate and carcass composition of red tilapia.

5. Conclusion

Microbial keratinase substances improve keratin protein hydrolysis and increases nutritive value of feather meal. Microbial hydrolysed protein is rich in essential amino acid contents and other oligopeptides which are good and cheap for poultry feed. Supplementary learning outcome is desirable in an integrated sustainable approach to solve environmental issue of keratinous solid waste management and provide cheap and healthy feed.

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Chapter 4

Evaluation of Protein Sources in Different Base Formulations Based on Total and Digestible Amino Acids for Japanese Quails

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Abstract

The Japanese quail (*Coturnix coturnix japonica*) rearing activity has grown considerably over the past few years. The purpose of this study was to compare diet formulations based on total (TAA) and digestible amino acids (DAA) and the use of different protein sources such as meat cum bone meal and feather meal in Japanese quails. The first three treatments were based on TAA, formulated based on corn and soybean meal (CST); meat and bone meal (CSMT); feather meal (CSMFT). The three other treatments received the same sequence of ingredients as the previous treatments; however, the diets were formulated based on digestible amino acids (DAA) (CSD, CSMD, and CSMFD). The results show that the base of diet formulation improves the performance of quails from 1-21d and the quails show that is possible to replacement of soybean meal with animal-origin by-products such as meat bone meal and feather meal, buts is needed to evaluate more aspects. In conclusion, diets formulated base on DAA for Japanese quails can be utilized with the same efficiency as diets based on TAA.

Keywords: amino acid, feed formulation, performance, protein sources

1. Introduction

The Japanese quail (*Coturnix coturnix japonica*) rearing activity has grown considerably over the past few years. Diversification of production in small and medium-sized properties, quick working capital, and reduction in production costs, compared with commercial layers, has intensified this growth.

The starter and growth phases of Japanese quails, from hatching to 42 days, is the critical time to manipulate these birds; within a short period, the quails become adults and are physiologically capable of laying eggs. Thus, any alteration in the nutrient levels or diet ingredients during this early phase significantly impacts the growth and development of the reproductive tract of birds. Diet formulation based on digestible amino acids (DAA) is a modern concept in poultry nutrition. This represents an advancement concerning diet formulation based on total amino acids (TAA) due to the greater reliability of the results in the assays of substitution of conventional feeds with feeds having more efficient protein deposition and of lower cost [1].

Nutritionists are now utilizing these formulations. This development is especially important due to the demand for optimizing the use of expensive raw materials and replacing corn and soybean meal with alternative ingredients, ensuring an equivalent uptake of DAA through the correction of deficiencies with supplementation of crystalline amino acids [2]. Formulation based on DAA, including meat and bone and feather meal, facilitates the absorption of greater amounts of lower quality protein sources and at lower costs, compared with soybean meal. Production costs are consequently lower, demonstrating the economic advantage of using this process. The cheapest diet formulation programs select feedstuffs that will produce the diet according to their price and nutritional value. From this perspective, the utilization of the formulation system based on DAA may be more effective than the formulation based on TAA as the nutritional value of the feedstuffs is better evaluated [3]. Thus, this study aimed to evaluate the effectiveness of diet formulations for Japanese quails based on DAA, compared with those based on TAA, using different protein sources.

2. Methodology of this research

The study was conducted in the Module of Poultry Science, in Areia-PB, Brazil. Located at latitude: −6.96179, longitude: −35.6953, 6 ° 57 ′ 42 ″ South, 35 ° 41′ 43 ″ West. All experimental protocols and procedures were approved by the Ethics Committee in Animal Experimentation (CEUA, n. 3363/18).

The first three treatments were subjected to diets formulated based on total amino acid (TAA), in which the first was formulated based on corn and soybean meal (CST); the second, in addition to corn and soybean meal, received meat and bone meal (CSMT); the third, in addition to the previously listed ingredients, received feather meal (CSMFT). The three other treatments received the same sequence of ingredients as the previous treatments; however, the diets were formulated based on digestible amino acids (DAA) (CSD, CSMD, and CSMFD). A completely randomized design was used with treatments.

A total of 576 female Japanese quails from one-day to 21 days (Starter phase, 1-21d) were distributed in six treatments with eight replications of 12 birds in each. A total of 480 female Japanese quails from 22 to 42 days (Growth phase, 22-42d) were distributed in six treatments with eight replications of 10 birds in each. A total of 288 female Japanese quails in the egg-laying phase were distributed in six treatments with six replications of eight birds in each, which period was divided into four cycles of 21 days, totaling 84 days.

For the formulation of diets, the nutritional requirements recommended by [4] and the total and digestible amino acid values of the ingredients recommended by [5] were considered. To calculate the DAA values, the TAA values were multiplied by the digestibility coefficients of the amino acids in the ingredients found by [5]. Daily management during the experimental period was based on feeding birds, washing drinkers, changing the water, cleaning the site, recording mortality, and collecting the orts to correct the calculations of the variables. The nutritional composition of experimental diets for Japanese quails from 1 to 21 and 22 to 42 days are presented respectively in (**Tables 1** and **2**). The nutritional composition of experimental diets for laying Japanese quails is in (**Table 3**).

Ingredients	Total amino acids			Digestible amino acids		
	CST	CSMT	CSMFT	CSD	CSMD	CSMFD
Corn	526.22	564.91	567.34	544.46	573.48	564.02
Soybean meal	441.89	393.74	377.00	426.67	383.80	385.09
Meat and Bone meal	—	26.47	25.35	—	26.65	25.54
Feather meal	—	—	15.85	—	—	11.97
Bicalcium phosphate	9.40	0.000	0.000	9.48	0.000	0.000
Limestone	5.46	4.95	5.24	5.52	4.98	5.16
Soybean oil	9.61	0.000	0.000	6.49	0.000	0.96
Salt	2.36	2.08	1.97	2.38	5.04	1.99
DL-Methionine	1.46	1.93	1.50	1.48	1.64	1.42
L-Threonine	0.60	1.34	1.02	0.52	0.77	0.44
L-Lysine.HCl	0.00	1.58	1.73	0.00	0.64	0.41
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Total	1,000	1,000	1,000	1,000	1,000	1,000
Nutritional Composition (g/kg)						
ME (kcal/kg)	2,900	2,900	2,900	2,900	2,900	2,900
CP, g/kg	250.3	249.4	249.5	249.2	249.2	249.7
Calcium, g/kg	6.0	6,0	6.0	6.0	6.0	6.0
Available phosphorus, g/kg	3.0	3.0	3.0	3.0	3.0	3.0
Sodium, g/kg	1.40	1.40	1.40	1.40	1.40	1.40
Lysine, g/kg	13.6	13.6	13.6	—	—	—
Dig. Lysine, g/kg	—	—	—	11.9	11.9	11.9
Methionine+cystine, g/kg	9.0	9.0	9.0	—	—	—
Dig. Methionine+cystine, g/kg	—	—	—	8.0	8.0	8.0
Threonine, g/kg	10.2	10.2	10.2	—	—	—
Dig. Threonine, g/kg	—	—	—	8.70	8.70	8.70
Tryptophan, g/kg	3.20	2.90	2.80	—	—	—
Dig. Tryptophan, g/kg	—	—	—	2.80	2.60	2.70
Arginine, g/kg	1.68	1.54	1.57	—	—	—
Dig. Arginine, g/kg	—	—	—	16.6	16.0	16.6
Valine, g/kg	11.50	10.60	11.20	—	—	—
Dig. valine, g/kg	—	—	—	10.0	9.80	10.30

[1]Vitamin mix composition basic of product: monoxide manganese, zinc oxide, iron sulfate, copper sulfate, calcium iodide, vehicle QSP Assurance levels per kg of product: 150.000 mg Manganese, Zinc 100,000 mg, Iron 100,000 mg, 16,000 mg Copper, Iodine 1,500 mg
[2]Mineral mix composition basic of product: Vit. A, Vit. D3, Vit. And, Vit. K, Vit. B1, vit. B2, Vit. B6, Vit. B12, Niacin, Folic Acid, Pantothenic Acid, Sodium Selenite, Antioxidant, Vehicle QSP Assurance levels per kg of product: Vit. The 10,000.00 U.I, Vit. 2,500,000 U.I D3, Vit. And U.I 6,000, Vit. K 1,600 mg Vit. B12 11,000, 25,000 mg Niacin, Folic Acid 400 mg Pantothenic Acid 10,000 mg, 300 mg Selenium, Antioxidant 20 g.

Table 1.
Nutritional composition of experimental diets for Japanese quails from 1 to 21 days.

Ingredients	TAA			DAA		
	CST	CSMT	CST	CSMT	CST	CSMT
Corn	629.48	647.49	661.72	627.8	643.64	664.88
Soybean meal	334.72	307.85	286.12	336.92	312.87	284.79
Meat and Bone meal	—	20.55	20.00	—	20.45	20.00
Feather meal	—	—	10.00	—	—	10.00
Bicalcium phosphate	7.35	0.00	0.00	7.34	0.00	0.00
Limestone	4.99	4.56	4.78	4.98	4.54	4.79
Soybean oil	13.23	7.97	5.63	13.75	9.18	5.46
Salt	2.48	2.25	2.18	2.47	2.24	2.18
DL-Methionine	1.74	2.01	1.84	1.62	1.66	1.71
L-Threonine	1.58	2.00	1.96	1.21	1.28	1.39
L-Lysine.HCl	1.43	2.33	2.77	0.91	1.13	1.80
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Total	1,000	1,000	1,000	1,000	1,000	1,000
Nutritiona Composition (g/kg)						
ME (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050
CP, g/kg	220.0	220.0	220.0	220.0	220.0	219.0
Calcium, g/kg	5.00	5.00	5.00	5.00	5.00	5.00
Available phosphorus,g/kg	2.50	2.50	2.50	2.50	2.50	2.50
Sodium, g/kg	1.40	1.40	1.40	1.40	1.40	1.40
Lysine, g/kg	12.0	12.0	12.0	—	—	—
Lisina dig, g/kg	—	—	—	10.5	10.5	10.5
Methionine+cystine, g/kg	8.30	8.30	8.30	—	—	—
Methionine+cystine dig, g/kg	—	—	—	7.40	7.40	7.40
Threonine, g/kg	9.60	9.60	9.60	—	—	—
Threonine dig, g/kg	—	—	—	8.20	8.20	8.20
Triptophan, g/kg	0.26	0.24	0.23	—	—	—
Tryptophan dig, g/kg	—	—	—	0.23	0.22	0.21
Arginine, g/kg	1.37	1.28	1.27	—	—	—
Arginine dig, g/kg	—	—	—	1.38	1.37	1.32
Valine, g/kg	0.96	0.91	0.93	—	—	—
Valine dig, g/kg	—	—	—	0.86	0.86	0.86

[1]*Vitamin mix composition basic of product: monoxide manganese, zinc oxide, iron sulfate, copper sulfate, calcium iodide, vehicle QSP Assurance levels per kg of product: 150.000 mg Manganese, Zinc 100,000 mg, Iron 100,000 mg, 16,000 mg Copper, Iodine 1,500 mg.*
[2]*Mineral mix composition basic of product: Vit. A, Vit. D3, Vit. And, Vit. K, Vit. B1, vit. B2, Vit. B6, Vit. B12, Niacin, Folic Acid, Pantothenic Acid, Sodium Selenite, Antioxidant, Vehicle QSP Assurance levels per kg of product: Vit. The 10,000.00 U.I, Vit. 2,500,000 U.I D3, Vit. And U.I 6,000, Vit. K 1,600 mg Vit. B12 11,000, 25,000 mg Niacin, Folic Acid 400 mg Pantothenic Acid 10,000 mg, 300 mg Selenium, Antioxidant 20 g.*

Table 2.
Nutritional composition of experimental diets for Japanese quails from 22 to 42 days.

Ingredients	TAA			DAA		
	CST	CSMT	CST	CSMT	CST	CSMT
Corn	553.47	569.45	601.31	563.58	575.89	619.98
Soybean meal	338.72	313.91	264.2	330.27	309.56	245.55
Meat and Bone meal	—	20.0	20.0	—	20.0	35.53
Feather meal	—	—	22.8	—	—	18.16
Bicalcium phosphate	13.05	5.88	5.43	13.09	5.9	—
Limestone	65.14	64.71	65.19	65.18	64.73	64.8
Soybean oil	20.32	15.48	10.15	18.58	14.7	5.6
Salt	4.79	4.57	4.41	4.8	4.57	4.27
DL-Methionine	1.47	1.72	1.33	1.5	1.52	1.6
L-Threonine	0.04	0.44	0.34	—	—	0.17
L-Lysine.HCl	—	0.83	1.85	—	0.13	1.34
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Total	1,000	1,000	1,000	1,000	1,000	1,000
Nutritional Composition (g/kg)						
ME (kcal/kg)	2,850	2,850	2,850	2,850	2,850	2,850
CP, g/kg	203	203	203	200	200	199
Calcium, g/kg	2.95	2.95	2.95	2.95	2.95	2.95
Available phosphorus, g/kg	3.5	3.5	3.5	3.5	3.5	3.5
Sodium, g/kg	2.3	2.3	2.3	2.3	2.3	2.3
Lysine, g/kg	10.8	10.8	10.8	—	—	—
Lysine dig, g/kg	—	—	—	9.5	9.5	9.5
Methionine+cystine, g/kg	7.8	7.8	7.8	—	—	—
Methionine+cystine dig, g/kg	—	—	—	7.0	7.0	7.0
Threonine, g/kg	7.9	7.9	7.9	—	—	—
Threonine dig, g/kg	—	—	—	0.67	6.7	6.7
Tryptophan, g/kg	2.5	2.4	2.2	—	—	—
Tryptophan dig, g/kg	—	—	—	2.2	2.2	1.9
Arginine, g/kg	13.5	12.7	12.4	—	—	—
Arginine dig, g/kg	—	—	—	13.3	13.3	12.6
Valine, g/kg	9.4	9.0	9.4	—	—	—
Valine dig, g/kg	—	—	—	8.3	8.2	8.3

[1]*Vitamin mix composition basic of product: monoxide manganese, zinc oxide, iron sulfate, copper sulfate, calcium iodide, vehicle QSP Assurance levels per kg of product: 150.000 mg Manganese, Zinc 100,000 mg, Iron 100,000 mg, 16,000 mg Copper, Iodine 1,500 mg.*
[2]*Mineral mix composition basic of product: Vit. A, Vit. D3, Vit. And, Vit. K, Vit. B1, vit. B2, Vit. B6, Vit. B12, Niacin, Folic Acid, Pantothenic Acid, Sodium Selenite, Antioxidant, Vehicle QSP Assurance levels per kg of product: Vit. The 10,000.00 U.I, Vit. 2,500,000 U.I D3, Vit. And U.I 6,000, Vit. K 1,600 mg Vit. B12 11,000, 25,000 mg Niacin, Folic Acid 400 mg Pantothenic Acid 10,000 mg, 300 mg Selenium, Antioxidant 20 g.*

Table 3.
Nutritional composition of experimental diets for laying Japanese quails.

In the phases of 1 to 21 and 22 to 42 days of age, the quails were housed in masonry pens with wood partitions. Each pen had a drinker and feeder for quails at the starter phase and heating was provided by incandescent light bulbs. Feed intake (FI) was calculated as the difference between the amount of feed supplied and collected orts, weighed at the beginning and end of each experimental phase. For the determination of weight gain (WG), birds were also weighed at the beginning and end of the experimental period. Feed conversion (FC) was calculated by dividing the accumulated FI by WG in the experimental period, adjusting the data by weighing feed and dead birds whenever there was mortality.

The economic evaluation of activity was performed during the entire experimental period through the relative gross margin (RGM), that is the gross margin (GM) of the diets formulated based on DAA concerning the GM of the diets formulated based on TAA. Similarly, the RGM of the diets with the inclusion of meat and bone meal and feather meal concerning the diet formulated based on corn and soybean meal was calculated. The determination of RGM was completed considering only the variable costs of feeding once the fixed costs were equal for all treatments. For these calculations, we considered the FI and the number of eggs laid by quails during the experimental period. Based on the price of inputs, it was possible to calculate the costs of each experimental diet per kilogram. The cost of feeding was obtained by multiplying this value by FI (in kg/bird). Gross income was calculated by multiplying the number of dozens produced in the period by the price of one dozen quail eggs in practice for retail in the Northeast region of Brazil. The GM of each treatment was calculated by the difference between the gross income and the cost of feeding.

The data were subjected to variance analyses using the computer package SAEG (Sistema para Análises Estatísticas e Genéticas, 9.1) and means were compared using a Tukey's test at 5% probability according to the statistical model $Yij = \mu + Ti + eij$, in which Yij is the response variable obtained in subject j, receiving treatment i; Ti is the effect of the additional treatment; eij is the experimental error associated with the additional treatment. The contrasts between the means of the variables obtained with the diet formulation based on digestible and total amino acids were also tested.

3. Results and discussion of this research

The performance results obtained from phase 1 to 21 days (**Tables 4** and **5**) show that there was a significant difference ($P < 0.05$) between the base formulations and the ingredients utilized only for FI in the diet based on corn and soybean meal (CS). Formulation based on TAA or DAA did not significantly influence WG; however, FC was higher with DAA. Birds during the starter phase deposited less dietary protein compared with older birds.

No effect of the formulation bases and ingredients utilized on performance was observed ($P > 0.05$) during the growth phase (**Table 6**). Likewise, neither the formulation based on TAA nor DAA influenced the performance values assessed. Evaluation of diets containing CS, CSM, and CSMF indicated no significant differences between the variables analyzed. Base formulations and ingredients utilized did not affect the performance of the quails ($P > 0.05$; **Table 7**).

Base formulations and the ingredients influenced the shell percentage significantly ($P < 0.05$) (**Table 8**). However, no significant differences were observed for any of the egg quality variables studied (**Table 9**).

The average results of the digestibility assay are presented in (**Table 10**). The diets that contained CSMFT presented greater nitrogen excretion.

	Ingredient		
Formulation basis	**CS[3]**	**CSMB[4]**	**CSMBF[5]**
TAA[1]	168.2 Aa	155.7 B	164.8 AB
DAA[2]	151.4 b	156.5	157.1
CV		3,38	

[A,B a,b] Means followed by different capital letters in the same row and different small letters in the same column differ statistically by the Tukey test at 5% probability.
[1]Total Amino acids.
[2]Corn and Soybean Meal.
[3]Digestible Amino Acids.
[4]Corn, Soybean Meal, Meat and bone Meal.
[5]Corn, Soybean Meal, Meat and Bone Meal, Feather Meal.

Table 4.
Effect of base formulations of different ingredients on feed intake (g) in Japanese quails from 1 to 21 days.

	Body weight (g/bird)	**Weight gain (g/bird)**	**Feed conversion (g/g)**
TAA[1]	70.7	63.4	2.596 A
DAA[2]	70.6	63.3	2.450 B
CS[3]	69.4 B	62.1 B	2.572
CSMB[4]	70.6 AB	63.3 AB	2.467
CSMBF[5]	72.0 A	64.7 A	2.489
CV	2.41	2.79	4.58

a,b Means followed by different letters in the same column differ by Tukey testat5% probability.
[1]Total Amino Acids.
[2]Digestible Amino Acid.
[3]Corn and Soybean Meal.
[4]Corn, Soybean Meal and Meat and Bone Meal.
[5]Corn, Soybean Meal, Meat and Bone Meal and Feather Meal.

Table 5.
Effect of base of formulation of different ingredients on body weight, weight gain and feed conversion in Japanese quails from1 to 21 days.

Base of formulationq	**Feed intake (g/Bird)**	**Body weight (g/bird)**	**Weight Gain (g/bird)**	**Feed conversion ratio (g/g)**
TAA[1]	393.3	158.7	93.2	4.22
DAA[2]	385.1	157.1	91.9	4.19
Ingredients				
CS[3]	387.7	159.1	93.7	4.14
CSMMSC[4]	389.8	156.3	91.1	4.28
MSCP[5]	387.7	158.1	92.9	4.20
CV (%)	5.78	3.55	6.07	5.06

[1]TotalAmino Acids.
[2]Digestible. Amino Acid.
[3]Corn and Soybean Meal.
[4]Corn, Soybean Meal and Meat and Bone Meal.
[5]Corn, Soybean Meal, Meat and Bone Meal and Feather Meal.

Table 6.
Effect of base of formulation of different ingredients in feed intake, body weight, weight gain and feed conversion in Japanese quails from 22 to 42 days.

Variables	TAA[1]	DAA[2]	CS[3]	CSMB[4]	CMBF[5]
Feed intake (g/ day)	25.494a	25.633a	25.938a	26.069a	24.684b
Egg production (%)	88.59 a	87.19a	89.55a	86.11b	88.02ab
Egg weight (g)	11.554	11.679	11.585	11.741	11.525
Egg mass (g/bird/day)	10.236	10.178	10.373	10.107	10.142
Egg mass conversion (g/g)	2.493ab	2.522ab	2.502ab	2.581a	2.438b
Conversion per dozen eggs (kg/dz)	0.345ab	0.351ab	0.348ab	0.363a	0.334b

[1]Total amino acids.
[2]Digestible amino acids.
[3]Corn and soybean meal.
[4]Corn, Soybean meal, meat and bone meal.
[5]Corn, Soybean meal, meat and bone meal and feather meal.

Table 7.
Performance characteristics of laying Japanese quails fed diets based on total amino acids (TAA) and digestible amino acids (DAA) and with different ingredients in this composition.

Base Formulation	CS[3]	CSMB[4]	CSMBF[5]
TAA[1]	7.53b	7.78 ab	8.01ab
DAA[2]	7.90ab	7.99ab	8.13 a
CV (%)		3.59	

[A,B a,b] Means followed by different capital letters in the same row and different small letters in the same column differ statistically by the Tukey test at 5% probability.
[1]Total amino acids.
[2]Digestible amino acids.
[3]Corn and soybean meal.
[4]Corn, Soybean meal, meat and bone meal.
[5]Corn, Soybean meal, meat and bone meal and feather meal.

Table 8.
Effect of base formulation and different ingredients in shell percentage (%) of laying Japanese quails[1].

	TAA[1]	DAA[2]	CS[3]	CSMB[4]	CSMBF[5]	CV(%)
Albumen, %	54.17	54.15	54.20	54.41	53.88	2.78
Height Albumen, mm	4.31	4.34	4.260	4.335	4.383	5.81
Yolk, %	33.09	32.53	32.95	32.46	32.99	3.66
Specific gravity (g/cm^3)	1.070	1.070	1.070	1.070	1.070	0.02
Shell Tickness, mm	0.274	0.268	0.264	0.273	0.278	5.91
Haugh Unity	87.89	88.59	87.84	88.21	88.66	1.70

[1]Total amino acids.
[2]Digestible amino acids.
[3]Corn and soybean meal.
[4]Corn, Soybean meal, meat and bone meal.
[5]Corn, Soybean meal, meat and bone meal and feather meal.

Table 9.
Egg quality of Japanese quails fed diets based on Total amino acids (AAT), digestible amino acids (AAD), and different ingredients in its composition.

The mean values obtained in the serum concentration of plasma uric acid (**Table 11**) show a significant interaction between the formulation bases and the ingredients utilized. CST diets differed significantly from the others, presenting lower plasma uric acid concentrations.

Varibless	Base formulation		Ingredients			
	TAA[1]	DAA[2]	CS[3]	CS[4]	MSCP[5]	CV(%)
N intake (g/bird/day)	0.728	0.762	0.746	0.721	0.715	11.61
N excretion (g/bird/day)	0.788b	0.768b	0.703b	0.625b	0.943a	12.77
N retention (g/bird/dia)	−0.059	−0.006	0.043	0.096	−0.228	15.78
Excreted N: N intake (%)	108.24	100.78	94.23	106.8	101.72	13.58
N retained: N intake (%)	−8.24b	−0.78ab	5.77ª	−6.80b	−1.72b	19.28

[1]*Total amino acids.*
[2]*Digestible amino acids.*
[3]*Corn and soybean meal.*
[4]*Corn, Soybean meal, meat and bone meal.*
[5]*Corn, Soybean meal, meat and bone meal and feather meal.*

Table 10.
Nitrogen balance and plasma uric acid of laying Japanese quails fed different bases and formulation ingredients in their compositions.

Variables	TAA[1]			DAA[2]		
		CSM	CSM		CSM	CSMF
	CST	T	FT	CSD	D	D
Starter diets cost (US$/kg)	0.435	0.42	0.42	0.43	0.415	0.415
		0.15				
Feed intake(1–21. kg/bird)	0.178	0.155	0.164	0.151	0.156	0.157
Cost of feeding (1–21. US$/bird)	0.07	0.06	0.06	0.06	0.06	0.06
Growth diets cost (U$/kg)	0.42	0.41	0.41	0.42	0.41	0.40
Feed intake (22–42. kg/bird)	0.387	0.4	0.392	0.388	0.379	0.387
Cost of feeding (22–42. US$/bird)	0.162	0.162	0.16	0.162	0.155	0.155
Laying diets cost (US$/kg)	0.40	0.39	0.38	0.39	0.385	0.37
Feed intake in laying stage (kg/bird)	2,185	2,076	2,031	2,087	2,020	2,079
Cost of feeding (84–133 days US$/bird)	0.88	0.82	0.77	0.83	0.78	0.77
Total feed intake (all stages)	2.74	2.63	2.58	2.62	2.55	2.62
Total cost feeding (US$/bird)	1.11	1.05	1.00	1.065	1.00	0.995
Egg production /BIRD	76.52	72.17	74.56	73.91	72.50	73.31
Gross income (US$/bird)	2.29	2.16	2.23	2.21	2.17	2.44
Gross margin (U$/bird)	1.18	1.11	1.23	1.145	1.17	1.44
Relative gross margin (%)	100	94.48	102.77	97.64	98.03	100.59

Table 11.
Production economic analysis of Japanese quails in initial stages, growth and laying stage.

The diets based on corn and soybean meal showed a slight elevation in cost about those that contained meat and bone and feather meals, regardless of the base formulations (**Table 11**).

Formulation diets based on TAA or DAA did not significantly influence WG; however, FC was higher with DAA, demonstrating that even if there is a remarkable increase in intake, diets formulated with DAA are more efficient at the utilization of the nutrients in this phase. Birds during the starter phase deposited less dietary protein compared with older birds. This may be associated with the low FI and reduced digestive efficiency due to the physical limitation of the gastrointestinal tract, which elevates the protein degradation rate to compensate for the high energy requirements of young birds [6]. The performance results of this phase also demonstrate that among the ingredients utilized, birds fed diet CSMF exhibited higher FI and WG than those on diet CS. Since proteins from animal origin by-products have a higher biological value and greater amino acid availability, there was a higher uptake of amino acids for the synthesis of muscle proteins; this is important for the development of this tissue. In the starter phase, the initial weight of Japanese quails increases seven-fold due to hypertrophy of muscles, the pectorals in particular, and the growth of bones and viscera [7]. However, Moura et al. [8] concluded that although the quail increases its body mass by approximately 10 times in the first 21 days, this growth is attributed to a greater synthesis of bone tissue more than muscle hypertrophy itself.

However, when utilizing animal-origin by-products in poultry diets, one must be very careful as to the quality of these ingredients and their nutritional compositions, since several factors can affect the amino acid availability. The digestibility coefficients of amino acids in meat and bone and feather meals can be influenced by the system in which they are processed, temperature, cooking time, drying, and the proportions of components within the product.

Shin and Vohra [9] demonstrate that Japanese quails reared for laying eggs exhibit a low tissue-deposition capacity after 35 days from birth due to the elevated mass of eggs produced daily. The early maturation of quails (35–42 days of age) leads to the need for feeding programs that maximize the growth rate and body development, which makes it possible for quails to reach the ideal weight at sexual maturity and ensures better uniformity of the flock during the laying phase [10].

According to Silva [11], birds during this stage are physiologically delicate since their body growth is impaired while the structures involved in egg production grow faster. Undernourished birds in this phase will be less productive, showing a shorter production peak and cycle at the laying phase. Important physiological alterations, such as an increase in the weight of the liver, ovary, and oviduct, are highly dependent on the level of available nutrients. Thus, diets formulated for growing Japanese quails must meet the requirements for them to absorb the nutrients necessary for the development of the reproductive tract, to be ready to begin laying eggs.

Formulations based on TAA significantly improved performance about higher FI compared with formulations based on DAA, however, no difference was identified for WG nor FC. This demonstrates that diet formulation based on DAA is more efficient at using nutrients in the starter and growth phases of Japanese quails. The maintenance of adequate body weight in layers during these initial phases is important since it interferes with the protein requirements of the bird. An adequate supply of protein for layers allows birds to reach sexual maturity with body weight and reserves capable of bearing the production phase without compromising the reserves [12]. This can be explained by the nutritional conditions established during the growth period influencing bird performance during the production phase. However, most previous studies have addressed the nutritional requirements of birds in the production phase, whereas research aiming to determine the requirements in the starter and growth phases is scarce [10].

Base formulations and ingredients utilized did not affect the performance of the quails ($P > 0.05$; **Table 7**). Wang and Parsons [13] results conform with the data presented here, with the diets for birds formulated with DAA performing equally as efficiently as those formulated with TAA. Evaluating the effect of ingredients utilized on bird performance indicated that diets containing CST exhibited higher FI and egg production. Diets with CSMT had higher consumption but lower production. In diets containing CSMFT, the FI values were the lowest recorded. However, their egg production was similar to the diets with CST. Freitas et al. [14] verified that quails display lower FI and egg weight with increasing metabolizable energy; however, they did not observe an influence of metabolizable energy on egg production. The average weight of a quail egg is 10.3 g, accounting for approximately 8% of the live weight of the bird; this is considerably greater compared with that of 3.5% in chickens. This indicates there is a major nutrients demand egg synthesis. Protein and energy content of the diet is a fundamental nutritional factor in egg weight determination [15, 16, 17]. Assessing the morphometric characteristics of the oviduct of quails fed diets of increasing protein levels, Artoni et al. [18] concluded that the birds receiving a diet containing 24%CP, the highest level, exhibited an increased thickness of the magnum gland, isthmus, and uterus. Consequently, egg weight and shell thickness increased. Furthermore, Ribeiro et al. [19] demonstrated that quails-fed diets containing 23% protein exhibited a greater FI, egg weight, egg mass, FC, and egg components (yolk and albumen) in comparison with the quails fed on 20%.

At 20% CP in the diets, bird performance remained stable for all the assessed variables when TAA and DAA base formulations were compared. Umigi [20] concluded that whilst in the laying phase, birds can have diet CP reduced by up to 5%without having impairing performance and egg quality, provided that the diets are properly supplemented with the essential limiting amino acids.

Base formulations and the ingredients used in the shell percentage significantly influenced egg quality (**Table 8**). No significant differences were observed for any of the egg quality variables studied (**Table 9**).

The results showed that diets based on DAA containing CSMFT were superior, demonstrating better use of the nutrients associated with the formation of the shell, such as calcium and phosphorus. Accordingly, birds can produce more resistant eggs with longer durability, allowing farmers to have better marketability of viable eggs. Similarly, the diets containing CSMFT are statistically superior in terms of this variable, demonstrating that the inclusion of these ingredients in the diet improves the quality of the shell of Japanese quail eggs. Dietary phosphorus (P) levels can alter the production and quality of eggs; however, the P level that promotes the highest shell quality may not be the one that promotes the highest egg production [21]. Plasma calcium (Ca) can exist when bound to the protein's albumin and globulin as well as when unattached, referred to as ionized (Ca2+). Half of the plasma Ca exists in the ionized form, and the other half bound to proteins. According to Ito [22], during the calcification period, the Ca deposited in the shell originates from two sources: dietary and from bones. Even if the birds consume an adequate amount of Ca from their diet, approximately30%–40% of the Ca deposited in the shell originates from the medullary bones. However, the utilization of Ca from the skeleton can be increased even with low levels of ingested Ca. The greater the dependence of Ca provision from the skeleton, the lower the amount deposited in the shell, thus resulting in eggs with thin shells and little resistance to cracking. The low-quality eggshell also presents a potential risk of bacterial contamination of the egg [23]. Among several factors that affect eggshell quality, the source and levels of Ca from the diet must be highlighted since the eggshell is almost totally comprised of calcium carbonate [22].

The average results of the digestibility assay are presented in **Table 10**. The diets that contained CSMFT presented greater nitrogen excretion. Such results can be explained by the low quality of the protein in the feather meal in these diets, which generated a reduction in the digestibility and absorption of amino acids. There were no significant differences between the retention of N by birds and formulation bases nor ingredients utilized. The excretion of N was higher than the intake in birds fed CSMFT and diets formulated based on TAA and DAA. This occurs in birds at more advanced ages due to the high desquamation of the intestinal epithelium, reducing the capacity of nutrient absorption.

That a reduction in protein content and the supplementation of the diet with industrial amino acids can substantially decrease N excretion. Excretion of N can be significantly reduced by an amino acid balance that meets the requirements of birds but with minimum excess, and especially through the supply of these nutrients in the digestible form [24].

The composition of excreta is directly influenced by the nutritional composition of the diet consumed by birds. Thus, when formulating diets with high CP levels based on TAA, with the utilization of a margin of safety or by the unfamiliarity of the nutritional composition of the feeds, a high protein content in the excreta is expected [25]. According to Lemme [26], the greater the inclusion of raw materials of low amino acid digestibility based on formulations of TAA, the less reliable the estimation of bird performance. This makes the poultry industry use an onerous margin of safety at the offer of nutrients. The largest portion of N excreted by birds is related to the material that was not digested and the excessive amino acids left after requirements for maintenance and egg production have been met [4].

CST diets differed significantly from the others, presenting lower plasma uric acid concentrations. This may be explained by the diets containing only CST having a higher amino acid digestibility than those containing the animal-origin by-products (CSM, CSMF), thus increasing the usage efficiency of these amino acids. Schmidt et al. [27] state that approximately 60%–80% of the uric acid excreted in birds is N. Uric acid is related to kidney and liver metabolism and is the main source of excretion of nitrogenous bases. Uric acid excretion is utilized as an indicator of protein quality ingested by birds; less protein is deposited in the muscle and more uric acid is excreted when birds receive low-quality protein in the diet, or when they are subjected to amino acid imbalance [28].

The diets based on corn and soybean meal showed a slight elevation in cost about those that contained meat and bone and feather meals, regardless of the base formulations (**Table 11**). This accrual may be attributed to the utilization of soybean meal being higher and the supplementation of industrial amino acids being lower in these diets to meet the amino acid requirement during this phase. The diets which contained the inclusion of the meat meals, however, resulted in a lower cost when compared with those using vegetable protein sources. The cost of the diet varied by 7.5% (US$0.03) and the cost of feeding had a variation of 11.94% (US$0.10). The impact on the reduction of soybean meal in the diet was 7.45%. In diet CSMFT, in which meat meal is included, the variation was 2%, whilst for the feather meal, it was 2.28%. In diet CSMFD, this inclusion was 3.35% and 1.81% for the meat and feather meals, respectively. The cost of feeding per bird varied by 10.85%, showing the lowest discrepancies in the diets that received the inclusion of the meals. Diet CSMFD was the most efficient.

The number of eggs produced during the laying phase (84 days) varied by 5.68% among the treatments, with a gross income at around US$2.58 per bird during the experimental period. The data on the relative gross income of the treatments were obtained through the percentage of the gross income of diet CST, formulated with corn and soybean meal and based on TAA. Diet CSMFT showed to be more efficient, reaching 1.94% over the relative gross income of diet CST. The relative gross income of diet CSMFD, however, was just 0.28% below that of diet CST.

4. Conclusions

Improved efficiency at FC was observed at the starter phase for the diets formulated based on DAA and when protein ingredients of animal origin were used. From 22 to 42 days of age, diets formulated based on DAA did not influence the performance of birds, appearing to be similar to those of the animals which received diets based on TAA. Diets formulated based on DAA for Japanese quails can be utilized with the same efficiency as diets based on TAA. The partial replacement of soybean meal by animal-origin by-products such as meat bone meal and feather meal for Japanese quails is possible, however, more research is needed to evaluate the inclusion of these ingredients in the different base formulations.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Essential Nanominerals and Other Nanomaterials in Poultry Nutrition and Production

Partha Sarathi Swain, Sonali Prusty, Somu Bala Nageswara Rao, Duraisamy Rajendran and Amlan Kumar Patra

Abstract

Poultry production, health and wellbeing are highly dependent upon formulation of balanced rations in terms of energy, protein, and micronutrients (vitamins and minerals). Among all, minerals are required in fewer quantities, but they are very important to maintain the productivity in poultry. Minerals present in the feeds are less bioavailable and additional supplementation is obligatory to meet the physiological demands of poultry. Conventionally, minerals are supplemented as inorganic salts, which are less absorbed and, thus, a major proportion is excreted to the surroundings creating environment issues. Nano-minerals and organic mineral chelates are other alternative to be used as livestock and poultry feed supplements. Though organic minerals are more bioavailable than inorganic salts, their high cost limits its use. In contrast, nano-minerals are relatively easy to synthesize at a lower cost. Nano-minerals are of the size from 1–100 nm and due to such small size, there is an enormous increase in surface area and thus their biological responses. The biological response studies have signified better retention of nano-minerals as compared to inorganic salts, and consequently leached less to the environment preventing possible pollution. Apart from these, nano-minerals have been shown to enhance growth, egg production and quality, immune-modulation and antioxidant status, and at the same time economize the production by reducing the supplemental dose of minerals and improving the feed conversion ratio. Some nano-minerals and other nanoparticles have strong antimicrobial effects, which have been shown to reduce pathogenic microorganisms in the gut. Nano-minerals seem to be less toxic than conventional mineral sources. Though less, few studies have indicated toxic effects of nano-mineral supplementation at higher dose of application, which should be validated by more programmed studies. Nanotechnology in poultry production system is still in its budding stage and more detailed studies are warranted to validate, establish and search for new effects of nano-minerals as they sometimes produce effects beyond expectation. This review highlights the biological responses of nanominerals on poultry production performance, quality of meat and eggs, tissue retention, immunity, antioxidant activity and antimicrobial actions compared with their conventional mineral sources.

Keywords: antimicrobial, health, nano-mineral, performance, poultry, quality, retention, toxicity

1. Introduction

Mineral nutrition is an indispensible part of animal feeding system which ensures optimum health, production, and reproduction in animals and birds. Even though, required in small quantities as compared to other nutrients such as energy and protein, their deficiency and imbalances are promptly reflected in the changes of animal wellbeing and their production. Sometimes, this may also cost the animals with their lives. They are essential for maintaining the normal health and productions; whereas in some cases additional supplementation could yield better growth and egg production. The significance of mineral nutrition is well documented and still new projects are undertaken to understand, explore better aspects and validate newer postulates associated in the field of mineral nutrition.

Conventionally, minerals are used in the diets through their inorganic salts, but low bioavailability of inorganic mineral salts necessitates using at higher doses in order to meet the animal requirements, which indirectly creates more pollution with minerals [1]. Recently, nano-sized minerals are considered to have greater bioavailability in animals and birds due to increased surface area, which tend to produce better desirable responses [2]. This chapter discusses synthesis of different nano-minerals, their mechanism of action, poultry performance, tissue retention, immunity, antioxidant activity and antimicrobial actions compared with their conventional mineral sources.

2. Importance of mineral nutrition in biological system

Minerals are vital for all biochemical functions in the body along with providing structural supports, electrolyte balance and homeostasis. The requirements of calcium (Ca) and phosphorus (P) in animals and poultry are comparatively greater than other minerals. They are mainly needed for bone development [3]. Zinc (Zn) is essential for several physiological and biochemical processes such as normal growth, reproduction, wound healing, ossification, DNA synthesis, cell division and gene expression, photochemical processes of vision, and augmenting the immune system of the body through lymphocyte replication and antibody production [1–4]. Selenium (Se) is essential for optimum animal production, fertility, and disease prevention [3]. However, role of Se in intra- and extra-cellular antioxidant systems is vividly recognized [5], which, as a component of glutathione peroxidase (GPx) neutralizes hydrogen and lipid hydroperoxide and thus maintains membrane integrity and guards from oxidative damage of lipid membranes [1]. Copper (Cu) is essential for normal growth, bone development, immune response, foetal development, nerve functioning, and in antioxidant system as a part or a cofactor of several enzymes [1]. Manganese (Mn) is an essential trace mineral necessary for optimum antioxidant, immune system as well as a component on several important enzymes [2]. Likewise, iron (Fe) is needed for synthesis of hemoglobin, which transports oxygen in the body and myoglobin, and is also associated with enzymes, e.g., peroxidases, hydroxylases, and catalase. Chromium (Cr) is a component of glucose tolerance factor and is essential for maintaining immune and antioxidant function and metabolism of lipids and proteins [6]. Combining all the effects together, minerals are associated with all the physiological functions in the body either involved directly or indirectly. Hence, a diet balanced in all the minerals is always a matter to maximize the productivity and health of the animals.

3. Sources of minerals

The minerals are present in all the food and feed sources as an integral part, but the amount required to support the productivity is not met through the feed resources. Added to this, the mineral component from plants are less absorbed and retained in the body as they form complex compounds with other components. For an instance, plant ingredients in the diets contain large amounts of unavailable P as phytates, which accounts almost 60–80% of total P and are not absorbed by the birds due to insufficiency of phytase enzyme [7]. Again, the bioavailability of minerals from traditional inorganic sources is relatively less for many minerals, while the requirements for high producing animals and birds are very high [8]. This necessitates the addition of minerals in the diets from extraneous sources [1, 9, 10], which gives the concepts of minerals as feed supplements in animal and poultry rearing. Minerals are generally supplemented in higher concentrations than their actual needs at cellular levels when inorganic supplements are added due to their poor bioavailability [11] along with chemical antagonism and interactions with other nutrients [12]. Conventionally, minerals are supplemented in the diets in their inorganic salts — oxides, sulphates, or carbonates — for instance, Zn oxide, Zn sulphate, sodium selenite, Ca carbonate, and dicalcium phosphate (DCP). The low bioavailable inorganic mineral salts supplemented at higher doses in order to meet the animal requirements, indirectly creates more pollution with minerals [1, 9, 10]. This issues needs to be addressed and better bioavailable mineral sources are a thrust of mineral studies for many decades. Many organic chelated minerals have been tried to fill the gap and reports indicated mixed responses considering their bioavailability, cost effectiveness and biological responses. Organic minerals as proteinate supplemented retained better in poultry signified better bioavailability as compared to their inorganic counterparts [13, 14]. Organic mineral supplementation has shown varied type of response in layers. For example, Rajendran et al. [15] reported improvement in laying percentage of birds, whereas Soni et al. [16] did not observed any effect on egg production by feeding organic minerals. In spite of better bioavailability of organic minerals over their inorganic counterparts, these sources are less used due to their higher cost [17]. This necessitates the urgent requirement for better bioavailable sources to be used particularly in poultry production to save guard the environment without affecting the animal or bird productivity at a cost effective manner.

Recently, nano-sized minerals are considered as a potential alternate to fill the gap and they have been tried and tested in many ways to validate their better bioavailability in diversified animals and birds. Nanotechnology confers the materials with particle size in nanometer (nm) range (<100 nm) at least in one direction, and by virtue of the nano-sized particle (NP), their structures exhibit significantly novel physical, chemical, and biological properties and functionality [18]. Due to their small size, the surface area increases many folds and thus they tend to produce many desirable responses [2]. The altered chemical and physical properties of NP could potentially modify the biological responses compared to its bulk materials [2, 19]. Studies have been carried out across the globe to unveil more beneficial effects as a feed additive in animals and birds, but still nanotechnology is in its infancy in the animal husbandry sector. In this chapter, we have tried to compile the various effects of the nano-minerals and other nano-materials in poultry.

4. Nano-minerals: synthesis

Nanotechnology deals with research and development related to nano-sized materials, and are specifically focused at understanding of measurement and

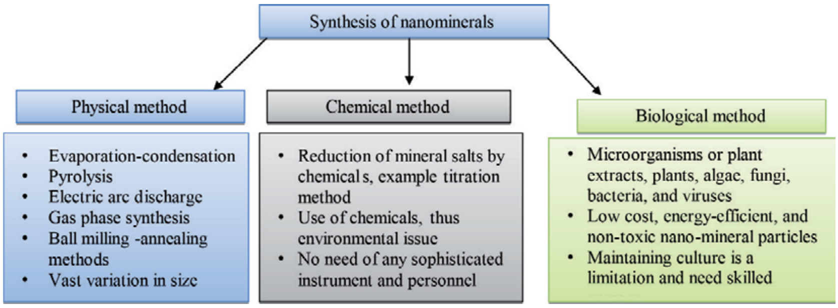

Figure 1.
Different methods of nano-minerals synthesis.

manipulation of matters at the nanoscales. Use of NP is gaining importance in diversified disciplines starting from medicine, environment, food, electronics, pharmaceutical applications, biotechnology, agriculture, and animal science [2]. Nano-minerals are specially synthesized mineral particles with its particle size ranging from 1 to 100 nm [20]. Like NP, nano-minerals possess higher physical activity and chemical neutrality, which may be a reason for efficient absorption in the animal system [21] and are reported to be stable under high temperature and pressure [22] as well. Nano-minerals as feed supplement can increase the feed efficiency, diminishing feed cost by reducing the supplemental doses, and simultaneously intensifying the yield and value of animal products by virtue of their superior bioavailability [1, 23, 24]. For example, nanominerals, due to their smaller size, were reported to be easily taken up by the gastrointestinal tract and efficiently utilized in vivo, and hence were more effective than the larger sized zinc oxide (ZnO) at lower doses [20]. Moreover, nanominerals exhibit lesser adverse effects as compared to their conventional counterparts. For instance, Reddy et al. [25] reported that nano-ZnO had less adverse effect on human cells. Nanominerals can cross the small intestine and further distribute into the blood, brain, and other different organs [26]. The functional properties of nanominerals, such as chemical, catalytic or biological effects, are highly influenced by their particle size, shape, composition, crystalline structure, surface ions, and morphology [27–29]. Nano minerals can be synthesized by physical, chemical or biological methods (**Figure 1**) [1, 19]. In physical method, physical forces are used to break down the larger sized materials to nanoscale, whereas in chemical method, reducing agents are used to reduce the particle size. Nanomaterials produced from physical method have wide range of particle size, but chemical method produce tentatively uniform particle size [19]. In biological method, also called green synthesis, different plant products or cultures are used for reducing the size of the intended materials. This method is free from use of corrosive chemicals which is the main constraint in chemical synthesis of NP. However, maintaining the culture needs technical expertise and is considered as a limitation in this method. Considering all points and methods, for use in livestock and poultry feeding, chemical method seems preferred as they are cheap, easy to produce and do not require any special instrument and expertise [19].

5. Mechanism of action of nanominerals

Nanoparticles are quite different in physical properties from bulk materials, contributing to wide range of new applications. Due to the much-reduced particle size they exhibit novel and improved physical, chemical, and biological activity that do not necessarily resemble the bulk mineral counterpart, and thus numerous modes of action are postulated by different workers. We have tried to compile the available resources keeping poultry nutrition in view. As such, further studies are warranted to establish or abolish any mechanism of action postulated till date.

Possibly the increased surface area of NP facilitates better interaction in biological interface and their increased retention time in the gut, reduced influence of intestinal clearance mechanisms and effective delivery of functional compounds to target sites result in better bioavailability and functionality [30]. By virtue of their small size, uptake by the gastrointestinal epithelium is much easier [20]. Uptake of NP through mucosal layer is dependent upon the charge on their surface and pH of adjacent environment. Changes in pH alter the surface charge and thus lead to agglomeration and change in size [31, 32]. For example, cationic NP was reported to be trapped within the glycosylated areas of mucin, whereas the diffusion of carboxylated anionic microparticles through the epithelial surface was better [33]. Nanoparticles are either absorbed through epithelial villi into the circulation and are subsequently transported to the liver and spleen [20, 34] or through M-cells in the Peyer's patches crossing the enterocytes and pass into the hepatic circulation [35]. Due to the smaller pore size (0.6 to 5 nm) of tight junctions, paracellular transport of NP is usually limited under normal physiological conditions [36]. Trace element NP may decrease mineral antagonisms in the intestine leading to enhanced absorption and utilization, thereby lowering their excretion into the environment. They are chiefly transported by transcellular mechanism. After absorption, their dispersion, breakdown, and discharge are related to their dissolvability, charge, and size. Nano-minerals have the potential to enter the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach after crossing the small intestine [26]. But their uptake rate in intestinal epithelia and other body tissues substantially differs [37]. The particle sizes less than 300 nm can reach to the blood circulation, whereas particles smaller than 100 nm can penetrate various tissues and organs [38].

6. Mineral absorption and metabolism

The amount of mineral absorbed and retained is termed as bio-availability, and this can be reflected by improved performance of animals or birds. Better bioavailability is indicated by more amount of mineral deposits in the organ, serum, and also better biological responses, and is affected by factors that influence absorption such as concentration, chemical forms, transport pathway, nutrient-nutrient interactions and excretory losses. Reports suggest that the bioavailability of inorganic salts is less, which results in high excretion of minerals into the environment through urine and feces [39]. Considering the other potential replacement of inorganic salts, organic and nano-minerals have provided encouraging biological effects when fed to animals and birds [1, 8, 21, 40, 41] with certain limitations.

Of the different mechanisms of transportation through intestinal epithelium, paracellular transport involves passage of substances across the epithelium through the intercellular spaces whereas transcellular transport involves passage of substances through the cells [42, 43]. Paracellular transport does not include any transporter or energy expenditure for transport and the absorption occurs along the concentration gradient, thus is not very efficient [42]. Tight junctions act as gatekeeper of paracellular transport and they exclude entry of macromolecules [42, 43]. Transcellular absorption involves either diffusion across concentration gradient or active carrier mediated transportation utilizing energy or through endocytosis [42]. Intestinal absorption can be improved by altering paracellular and transcellular transport. Compared with CuSO4 and CuO microparticles, CuO NP are believed to be rapidly transported into cells, and subsequently interact with the Cu transport proteins [44], with a non-antagonism of CuO NP with Zn. Na et al. [45] suggested the possibility that CuO NP are absorbed through a different pathway that other Cu sources use [1].

7. Nano-mineral supplementation and mineral retention

Liver handles most of the absorbed nutrients and regulates their release into blood circulation for further distribution in different tissues or excretion. Hence, increased concentration in liver is a suitable indicator of retention status. The minerals supplemented in their NP forms increase their bioavailability and utilization efficiency [46]. Minerals supplemented in their nano-forms retained better in in vivo studies as compared to their inorganic salts [41, 47, 48]. Patra and Lalhriatpuii [1] extensively reviewed the retention of nano mineral supplementation in poultry and suggested that Ca, P, Zn, Cu, Se, Mn and Fe in their nanoforms are retained better than their inorganic counterpart. Owing to better bioavailablity, Ca and P supplementation as nanominerals reduces the quantity of supplementation, thus making the ration economic and environment friendly [1]. Sohair et al. [49] reported that the use of hydroxyapatite NP is economically efficient as compared to the control diets. Nano-Zn in lower dose could be a good substitution in mineral premix instead of ZnO, which tend to improve carcass characteristics and oxidative stability of chicken meat [50]. Hu et al. [51] studied the selenium retention from nano-Se and selenite origins in chickens by the intravenous or oral administration of the radio labeled ^{75}Se and the in vivo ligated intestinal loop procedure, and reported higher nano-Se retention in the whole body and liver tissue compared to that of selenite, and intestinal transport of Se through ligated intestinal lumen loop to body was higher than that of selenite. Retention of Se is found to be influenced by the dietary Se source and concentration of Se supplemented as well. Reports suggests nano-Se supplementation to Guangxi Yellow broilers diet improved hepatic and muscle Se contents in a dose dependent manner up to a supplemental dose of 0.3 mg/kg [52], suggesting a relationship between nano-Se metabolism and liver function. Similar responses were also reported by Meng et al. [53] and Mohapatra et al. [54], where they obtained higher Se deposition efficiency in nano-Se supplementation groups than sodium selenite group, which also suggests better retention of nano-Se than that of sodium selenite as suggested by Zhang et al. [55]. Radwan et al. [56] observed higher Se content in eggs by nano-Se supplementation as compared to sodium selenite, most probably due to the faster transfer of nano-Se into the egg. A linear and quadratic increase in liver and muscle Se in a proportionally to the dietary nano-Se level, with a the peak value at 2.0 mg/kg of dietary nano-Se was reported; however, considering meat quality, immune function, oxidation resistance, 0.3 to 0.5 mg/kg was reported to be the optimum level of supplementation of nano-Se for broilers [57]. Supplemental nano-Cr picolinate at 0.5 and 3 mg/kg of Cr increased Cr and Ca concentration in the liver and egg, and improved Zn and Mn retention in layer chickens [58]. Nano-Cr added at 0.4 mg/kg feed was found to increase the retention of Cr, Ca, P, Zn, and Fe in layers, increased the Cr and Zn concentration in plasma, liver, and eggshell; zinc in egg yolk; Ca in the liver and eggshell [59]. However, no increase in Cr content in the eggs and blood of Japanese quails was reported due to dietary addition of 0.2 to 0.8 mg/kg of nano-Cr [60]. Jankowski et al. [61] reported no effect of reducing Mn from 100 to 50 or 10 mg/kg either from NP-Mn2O3 or MnO on the growth performance or oxidation process in liver and breast muscles and increased Mn accumulation and reduced Zn and Cu accumulation in the liver, breast muscle and skin but increased intestinal absorption of Zn. Nano $MnSO_4$ supplementation resulted in improvement of tibia bone characteristics such as tibia length, tibia volume, tibia breaking strength, tibia diameter and bone weight [1]. Intramuscular injection of Fe-NP at 2 mg/kg BW improved body weight gain, hematological traits, and tissue retention of Fe in broiler chickens [62]. The enrichment of the food with nano-Fe improved Fe concentration in the body (by 5.3%) and erythrocyte in blood [63]. Cysteine-coated Fe_3O_4 NP at 1.2 mg/kg diet recorded similar weight gain, feed efficiency, hematological and biochemical parameter as that of 120 mg/kg of $FeSO_4$ in a quail diet [64]. Overall, the above observations suggest that different mineral NP usually increased retention of minerals in tissues, eggs and meat even at the lower doses compared with their conventional sources of minerals.

8. Growth performance and meat quality

Most of the studies on poultry has emphasized on growth promoting effect as well as mineral retention due to supplementation of nanoforms of minerals (**Figure 2**). Studies have shown growth promoting effects by feeding nano-Zn [48, 50, 65, 66], nano-Se [52, 54, 67], nano-Ca [49, 68, 69] and nano-Ag [70]. Mohammadi et al. [71] observed improved growth performance in broilers supplemented with nano-Zn-methionine and nano-Zn-max at 80 mg/kg of diet; however, dietary nano-Zn sulphate reduced growth performance in broilers. Nano-Ag supplementation at 4 mg/kg caused an improved body weight gain and best feed conversion ratio in broiler [70]. Silver acting as an antimicrobial agent on intestinal harmful bacteria may improve gut health leading to better nutrient absorption, which was manifested by improved weight gain, feed intake and feed conversion ratio of broilers fed diets containing Ag NP [72]. Nano-Ca phosphate at 50% level of recommended supplementation resulted in improved body weight gain without altering feed conversion ratio, carcass characteristics and biochemical parameters similar to the 100% DCP supplemented group [73].

Supplementation of DCP NP has been reported to show better feed conversion ratio and body weight gain in poultry at 50% levels [68] and at 1.75, 1.31, and 0.88% levels [69] when compared to the control groups fed with larger DCP particles. Similar to DCP NP, hydroxyapatite NP also increased growth rate and feed intake in broilers at 2 to10% supplementation [49] without affecting the digestibility coefficient of other nutrients. However, Sohair et al. [49] observed that supplementation of 0.12% of calcium phosphate-NP instead of the conventional DCP at 2% resulted in better body weight gain, feed efficiency and economic efficiency

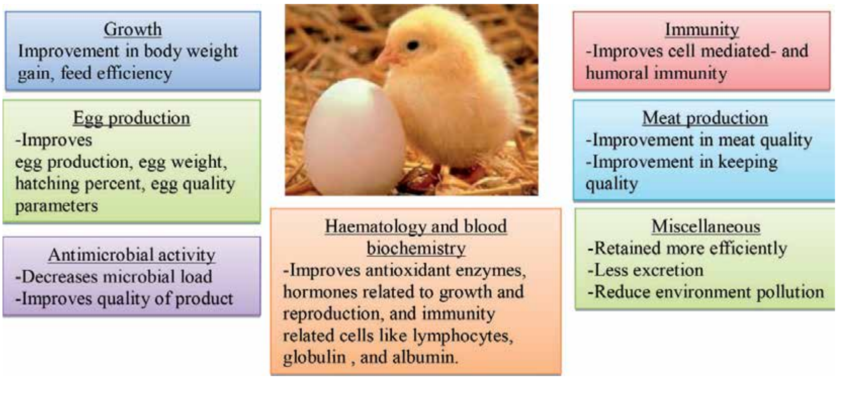

Figure 2.
Different beneficial biological effects of nano-minerals in poultry.

in broiler chickens. Mishra et al. [48] fed layer birds with extremely low levels of nano-Zn (1/500th of basal dose) and obtained similar growth promoting effect. Likewise, Ahmadi et al. [65] observed increased body weight gain, feed intake, and feed efficiency at 60 and 90 mg/kg diet of ZnO NP, but exhibited a lower performance in chickens at a dose of 120 mg/kg diet, whereas Fathi [66] reported lower feed efficiency in birds by supplementing nano-ZnO at 40 mg/kg. In contrast, feed intake, body weight gain, feed efficiency and, carcass traits were not affected due to supplementation of nano-ZnO at 25 and 50 mg/kg as well as ZnO at 100 mg/kg [50]. Nano-Zn at 80 mg/kg increased Zn, Ca and P levels, bone dimensions, weight, total ash along with higher liver and muscle Zn concentration [74]. Significantly lower fat and cholesterol content and better antioxidant status was obtained by supplementation of nano-Zn than inorganic Zn at 80 mg/kg [74]. Nano-ZnO increased mRNA expressions of insulin like growth factor-1 and growth hormone genes in broiler chickens compared to the inorganic or organic Zn [75].

Nano-Cr increased protein contents in thigh and breast muscles and lowered fat and cholesterol concentrations in thigh muscles. Chromium NP at 0.5 mg/kg diet improved breast and thigh muscle protein content, average daily gain and feed efficiency and lowered cholesterol and fat in thigh muscles of the broilers [76]. However, supplemental nano-Cr picolinate at 0.5 and 3 mg/kg [58] or 0.4 mg/kg [59] of Cr did not affect body weight, feed intake, feed efficiency, and egg production of layer birds. Supplementation of nano-Cr at 0.8 mg/kg diet was effective in reducing the negative effects of induced stress on meat quality of quail broilers, as evident from reduced malondialdehyde concentration in the thigh muscle and unaltered hematological parameters [77]. CuO NP was more efficient in increasing growth performance and immunity compared with the conventional sources of Cu [78]. As compared to the inorganic salts, supplementation of nano-Se improved the body weight gain and lowered the feed to gain ratio at doses up to 0.30 mg/kg diet, beyond which no beneficial effect was recorded [52, 54]. Se-yeast and nano-Se resulted in better growth performance than sodium selenite at 0.2 mg/kg Se [79]. Nano-Se supplementation reduced drip loss percentage without altering weight gain, feed intake, and feed conversion, meat color or immune organ index (thymus, bursa, and spleen) in broilers [57]. However, Se supplementation irrespective of its source (sodium selenite or nano-Se) increased daily weight gain and feed efficiency [67], though nano-Se proved to be more efficient. Improved weight gain and feed conversion due to supplementation of Fe NP (7 mg/kg) was possibly due to increased arginine in liver [80]. Dietary Fe sulfate NP resulted in highest jejunal villi width and surface area in broiler chickens at 21 and 42 days of age [81]. Overall, above studies that nano-minerals at reduced dose rates have potential to improve growth performance and the quality of meat compared with the conventional mineral sources.

9. Impact of nanominerals on layers

Eggs and meat are the primary products of poultry industry which are widely accepted, consumed and provides the quality nutritional security to the human race. The huge demand of poultry products intensifies the production system and improving the productivity becomes the major focus of research. The composition and nutrient density of poultry diets affect composition and nutritional quality of eggs and their products [82]. Considering the layer birds, precise mineral nutrition is very important to maintain the egg production and egg production is very susceptible to minute deficiency of many minerals. Several studies have been conducted to validate the effects of nanominerals in layers and their effects on egg productions are documented.

Calcium is required for egg shell formation; hence its requirement in layer diets is very high to sustain egg production. On adding 1 g/L of Ca carbonate NP in water, Wang et al. [83] could find stronger eggshell strength and better freshness indexed eggs as compared to the control. However, Ganjigohari et al. [84] reported a drop in egg production percentage, egg mass and low blood Ca level by reducing 4.03% of Ca carbonate by 0.126% Ca carbonate NP in laying hens as compared to birds supplemented with 4.03% of Ca carbonate replaced by 2.02%, 1.01%, 0.25% Ca carbonate NP, which would be due to too much reduction in the Ca level in the bird's diet. Similarly, Zn supplementation is obligatory to improve egg production and quality [1]. Being a part of enzyme carbonic anhydrase, Zn is required during egg shell formation and thus deficiency of Zn results in poor eggshell quality. Zn also interrelate Ca crystals during eggshell synthesis [85] which indirectly affects formation of shell membrane and eggshell. Abedini et al. [85] reported an improvement in feed intake, egg mass, egg Haugh unit, eggshell thickness and strength, and tibia ash content and strength at 40 and 80 mg Zn/kg diet as ZnO NP in the laying hens. Tsai et al. [86] observed a hike in Zn retention, thickness of the eggshell, concentration of growth hormone in the blood serum and carbonic anhydrase in nano-Zn supplemented groups as compared to control, without affecting the immunity and other nutrient retention in birds. However, no effect of different dietary Zn sources such as inorganic, organic or nano-Zn on egg quality parameters namely, egg mass, eggshell weight, and eggshell breaking strength [87, 88]. An increase in Zn content in egg yolk due to supplementation of nano-Zn may be proved advantageous to produce designer egg and aid in better keeping quality [82]. Olgun and Yildiz [89] observed highest egg weight and the lowest eggshell thickness by supplementation of nano Zn at 50, 75 and 100 mg per kg diet as compared to Zn-sulphate and Zn-oxide and Zn-glycine supplemented birds and thus, suggested nano-Zn supplementation negatively affects the eggshell thickness and bone mechanical properties. However other studies reported positive responses by supplementation on nano-Zn [82, 85]. The malondialdehyde content in egg was reduced in the groups supplemented with Zn-oxide NP at 80 mg Zn/kg diet [87] and 40 mg/kg of Zn-oxide NP [66], respectively. Dietary Se has a significant role in egg production and immunity in poultry and reports suggests better responses of nano-Se as compared to its conventional counterparts. Radwan et al. [56] reported that use of nano-Se improved Se content in eggs, egg production and feed conversion ratio without affecting egg weight, feed intake in birds. Qu et al. [90] supplemented Se-NP at 0.5 mg/kg diet in laying hens and reported improved rate of egg production, glutathione peroxidase (GPx) activity, total antioxidant status, along with decreased soft-shelled or cracked egg rate. Meng et al. [53] observed an increase in egg Se concentration by supplementation of Nano-Se at 0.3 mg/kg as compared to sodium selenite, Se-yeast. However, they observed that Se-yeast and nano-Se were better retained in the body than sodium selenite. It is observed that injection of nano-Se and nano-ZnO lessened the negative effects of heat stress by increasing antioxidant activity and reducing oxidative stress [91]. Shokraneh et al. [91] observed increased activity of GPx and superoxide dismutase (SOD) and total protein and decreased the levels of corticosterone, cortisol, T4 and T3 in the eggs being injected with NaCl solution containing 40 μg nano-Se at high eggshell temperature. In ovo injection of Cu-NP on 1st and 10th day of incubation showed decreased oxygen consumption, lower heat production, higher residual yolk sac weight compared with the control group, signifying reduced lipid oxidation in Cu-NP injected group [92]. Supplementation of nano-Cu in poultry has shown varied effects depending on dose administered. For an instance, in ovo supplementation of nano-Cu and Cu-sulphate NP at 50 mg hiked the red blood cells and white blood cells in poultry [93], while at a dose of 0.3 mL containing 50 mg/L nano-Cu improved the expression of pro-angiogenic and pro-proliferative genes [94], and also improved the bone characteristics at 42 days birds which signified the possible effect of nano-Cu in bone development and maintenance [95]. The better performances in poultry by in ovo injection of nano-Cu was attributed to improved metabolic rate during embryogenesis resulting in amplified performance of broiler chickens after hatching [96]. Supplemental nano-Cr-picolinate at 0.5 and 3 mg/kg of Cr improved egg quality, retention of Cr and Zn, whereas, it decreased shell ratio in the 60th day eggs [58]. Nano-Cr supplementation at 0.4 mg/kg of feed also improved egg quality parameters including Cr and Zn content in eggshell, Ca in the liver and eggshell, and Zn in egg yolk [59]. Another study specified that eggs of birds supplemented with nano-Cr at 0.2 and 0.4 mg/kg feed exhibited higher shell strength than eggs of hens from the group control and receiving Cr in an inorganic form [97]. In ovo supplementation of nano-Fe though improved body weight gain to egg ratio and feed conversions ratio at 7 days of age in broiler chicks, but no such improvement was observed later and there was no significant effect on hematological parameters [98]. L-cysteine-coated Fe-oxide NP at 6 or 60 mg/kg diet improved availability and utilization of Fe as evident from increased percentage of egg production and egg weight in quails [99]. Taken together, nano-minerals can improve egg production and s eggs quality traits at decreased rate of application.

10. Impact of nanominerals on anti-oxidative activity

Trace minerals particularly Zn, Se, Cu, and Mn play a major role in anti-oxidant system, either being a component of the antioxidant metallo-enzymes or indirectly by regulating their activities. These enzymes act an indicator of the wellbeing of the animals and they increase or decrease depending on the mineral level in the animal or birds. Hence, better bioavailability of a mineral source can be determined by monitoring these enzyme levels which are mineral specific. Different mineral NP have been shown to improve antioxidant status in birds compared to their conventional forms (**Figure 3**). Supplementation of ZnO-NP at 40 and 80 mg/kg in the diet of broiler chickens augmented their antioxidant status as evidenced from increased activity of SOD and catalase, and decreased concentration of malondialdehyde [100]. Zhao et al. [17] observed higher total antioxidant activity in serum and liver tissue, serum catalase activity and reduced serum and liver malondialdehyde concentration in the 20 mg/kg nano-ZnO group of broiler chickens

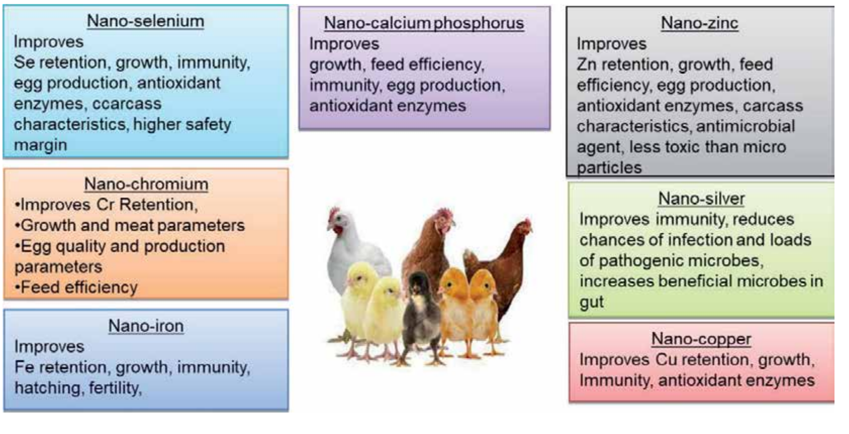

Figure 3.
Beneficial effect of different nanominerals in poultry production.

compared to the control (60 mg/kg ZnO) chickens. Zhao et al. [17] also reported higher activity of serum and liver Cu-Zn-related SOD and serum catalase in the diets containing 60 and 100 mg Zn/kg from ZnO NP. Bami et al. [50] reported that ZnO NP at 25 and 50 mg/kg as well as conventional ZnO at 100 mg/kg did not affect feed intake, body weight gain, feed efficiency and, and carcass traits, but higher dose of ZnO NP lowered malondialdehyde content and cooking loss in meat compared with 100 mg/kg of ZnO. Lina et al. [101] also observed increased GPx activity, total antioxidant activity in serum and reduced serum malondialdehyde content in Arbor Acres broiler chickens at 40 mg/kg of Zn supplementation from nano-ZnO. At higher levels (80 mg/kg) of Zn supplementation through either green nano-Zn or commercial nano-Zn in broiler birds showed significantly higher serum SOD, GPx, and catalase levels than their 40 and 60 mg/kg diet and inorganic Zn at 40, 60 or 80 mg/kg diet [74]. Ahmadi et al. [65] observed significantly increased SOD activity at 60–90 mg Zn/kg diet from ZnO NP in broiler chicken ration compared to 30 mg/kg diet. However, they observed inhibitory action of nano-Zn at further increased level (120 mg/kg diet) apparent from decreased SOD activity. Supplementation of 0.19 mg Se/kg diet from Se-NP increased serum SOD and GPx activity and decreased malondialdehyde concentration compared to 0.15 mg Se/kg diet from coarse Se in broiler birds [102]. Nano-Se at 0.3 mg/kg diet expressed improved GPx activity, free radical inhibition, immunoglobulin M concentration and decreased glutathione and malondialdehyde content in serum, improved GPx activity and free radical inhibition in liver and GPx activity in muscle [57]. Elkloub et al. [70] observed better total serum antioxidant activity in Ag-NP supplemented groups (2, 4, 6, 8, 10 mg/kg) and best in 4 mg/kg diet in Ag-NP supplemented group than un-supplemented group. Jankowski et al. [103] observed no adverse effect on antioxidant defense on reducing Mn concentration from 100 to 10 mg/kg diet in the form of NP-Mn_2O_3 whereas a 50% reduction in Mn level in the form of MnO enhanced lipid oxidation processes in turkeys.

11. Effect of nanominerals on health and immunity

Trace essential minerals also act as immune stimulants in birds. Hence balanced mineral mixture is given much priority to maximize the animal or bird productivity and minimize the stress (biotic as well as abiotic) in animals and birds. Minerals particularly Zn, Cu, Se and Mn are studied on their immune-stimulant effects profusely, which may require at higher concentrations for better immunity compared with the optimum production levels [1, 2].

Different nanominerals have showed to exhibit better immune responses (**Figure 3**). Hafez et al. [100] observed enhanced cellular immunity evidenced from increases in serum IgY concentration, total lymphocyte count, macrophages, phagocytic activity and phagocytic index in ZnO-NP fed groups compared to ZnO supplemented group. Nano-Zn supplementation at 0.06 mg/kg in the basal diet improved immune status of broiler equivalent to that of 15 mg/kg diet of organic Zn supplementation in term of increased weight of lymphoid organs and improved humoral immunity [47]. Supplementation of ZnO-NP in dry broiler ration improved carcasses yield and relative weight of lymphoid and digestive organs compared to wet diet during the starter period [104, 105]. Retention of Se in liver and muscle increased in a dose dependent manner with dietary intake of nano-Se (0.3, 05, 1 and 2 mg/kg diet), but did not affect growth performance whereas improved meat quality, immune function, and oxidation resistance were observed for nano-Se level ranging from 0.3 to 1 mg/kg diet [57]. Addition of Cu-NP in drinking water (10 mg/l) improved immunity, and productivity more efficiently compared to coarse CuSO4 [106]. A study involving Cu-NP, agglomerates of Cu-NP and Cu microparticles on the metabolism in broiler chickens after a single intramuscular injection revealed that all these forms had growth stimulating effect along with increased red cell level, hemoglobin, Cu and protein in blood serum, where effects were expressed quickly by Cu-NP [107].

12. Nanominerals as antimicrobial feed additives

Pathogenic microbial load in the gut of poultry is detrimental as they reduce growth rate, feed efficiency and mortality, and are some of these contaminants may survive during food processing and storage. The in-feed antibiotics used for preventing the pathogens as well as growth promoters has been great concern due to possible emergence of drug resistance in microbes as well as appearance of the drug residue in poultry products and subsequently affecting consumer's health [108]. There is an optimism of using nano-minerals as antibiotic alternatives due to their antimicrobial properties [109]. NP use has been established in therapeutics, drug delivery and diagnostics [110].

Many research carried has explored the antimicrobial action of metal oxide-NP [109]. Nano-Ag supplementation at 4 mg/kg diet in broiler chickens reduced serum cholesterol, aspartate aminotransferase levels and reduced caecal *Escherichia coli*, but had no significant effect on Lactobacillus count [70]. Nano-Ag in water (25 mg/kg) increased the population of lactic acid bacteria without any adverse effect on enterocytes of duodenal villi [111]. Nano-Ag lowered the number of *Escherichia coli*, *Streptococcus*, *Salmonella*, and total mesophilic bacteria in the litter [112]. ZnO-NP was found be effective against both gram positive and gram-negative bacteria [109, 113] as well as spores that are resistant to high temperature and pressure [29]. ZnO-NP are also effective in inhibiting the growth of fungi (*Aspergillus flavus*, *A. ochraceus* and *A. niger*) and their mycotoxins production [114]. Some possible mechanisms of bactericidal action of metal oxide NP was generation of reactive oxygen species inside the bacterial cells (e.g., hydroxyl, hydroperoxide, and superoxide radicals) that may damage lipid membranes of cells and organelles of bacteria [109, 115] or they alter permeability of bacteria after entering their plasma membrane resulting in cell death [116]. They may damage bacterial cell after penetration by interacting with sulfur and phosphorus containing important compounds like DNA [113]. As per Rajendran et al. [117], ZnO NP inactivates the proteins that are responsible for transport of nutrients, thus decreasing the membrane permeability and eventually causing the cellular death. Another explanation about antimicrobial action of metal oxide NP is that microorganisms carrying a negative charge are electromagnetically attracted towards metal oxides carrying a positive charge, subsequently leading to oxidization and death of microbe [113]. The antibacterial activity depends on the size, with better result obtained with smaller size [118]. But, Arabi et al. [113] observed the significance of surface area and concentration of NP, whereas crystalline structure and shape of NP have little significance.

13. Environment implication of nanomineral supplementation

In high intensified production system, trace minerals are added to poultry diets in high amounts exceeding the birds' requirements, with a large safety margin, creating environmental issues particularly in areas of intensive poultry production [8]. A study reported that by using poultry manure, Zn content in soil was found in excess by 660% in comparison to plant Zn requirements, predisposing to phytotoxicity [119]. Among minerals, Fe, Cu and Mn are always found in excess than the amount required by the plants [8].

Poor retention efficiency on inorganic mineral sources leads to excretion of unabsorbed minerals to the environment, which may be a potential environment pollutant especially in the area of intensive poultry farming. Phosphorus excretion in poultry excreta from large scale poultry farming has been a matter of concern from environment pollution point of view. Predominantly, inorganic P sources are used in poultry ration for optimum growth, egg production which results in excretion of huge amount of P to the environment [1]. Improved absorption of minerals through NP may reduce excretion of unutilized minerals and could minimize the environmental pollution. Cufadar et al. [88] reported that the Zn content of the feces was less in poultry when they are supplemented with nano-Zn as compared to organic and inorganic Zn. Furthermore, Hassan et al. [69] found that DCP NP supplementation reduced excretion of Ca and P by 50.7 and 46.2%, respectively. Dietary Cu is absorbed in a range between 10 to 30% in the small intestine of the animal creating environmental issues [3, 92], but supplementation of nano-Cu has been proved effective in prevention of environmental leaching of unabsorbed Cu by virtue of its efficient intestinal absorption and also functions at the molecular level [120, 121]. Considering Zn, predominantly ZnO or ZnSO4 are used in commercial poultry feeds and among these, ZnO is used in 80–90% cases having less bioavailability [122]. Reports suggests nano-Zn are better absorbed in different animals [40, 41] and birds [47, 48], thus reducing the amount of Zn excreted to the environment, and environment pollution. Reports suggest that nano-Cr has better bioavailability than organic and inorganic Cr supplements [123]. Therefore, nanominerals offer opportunities to reducing environmental pollution of minerals without compromising the production, health and quality of products.

14. Special aspects of nanominerals

Trace minerals such as Se is essential in minute quantities in poultry diet to reduce stress, improve immunity and overall health, but safe limit of inorganic Se is very narrow. Hu et al. [51] observed a wider range between the optimal and toxic dietary levels of nano-Se compared to inorganic sodium selenite in broiler chickens. But contradictory reports exist regarding occurrence of severe pathological changes in liver due to increase in nano-Se concentration from 0.15 to 0.3 mg/kg diet [124]. One of the possible mechanisms of nano-Se action in poultry could be conversion of nano-Se into selenite, H2Se or Se-phosphate followed by synthesis of selenoproteins by gut microbiota, which was also reported by Surai et al. [125] in *Veiollonella*. Reports suggest lower toxicity of Se NP than selenomethionine [126]. Also, Gangadoo et al. [127] reported that nano Se did not cause any damage to epithelial cells in the digestive system and neuronal bodies in brain tissue signifying its lesser toxicity in animal models. Nano-Ag in higher concentration (8 and 12 mg/kg diet) had detrimental effect on organs such as liver [128]. Ag-NP supplementation in drinking water (15 mg/L) of broilers had no significant growth promoting or coccidiostat action [129]. At higher concentration (50 mg/L) in drinking water, no effect on the intestinal colonization of C. jejuni was found; however, reduced body weight gain was observed in broiler chickens [130]. Dietary ZnO NP at 40 and 60 mg/kg alleviated the negative results of heat stress evident from lowering of serum corticosterone level [131]. Saki and Abbasinezhad [132] reported improved embryonic growth and development in broiler on supplementation of 25 mg/kg diet of nano-Fe and 100 mg/kg diet of Fe-nano-alimet chelate. But toxicity of nano-FeO was reported in chick embryo possibly due to its interaction with egg albumen.

FeO NP caused 100% mortality at 200 mg/L and decreased body weights and crown-rump lengths of embryo at 50 and 100 mg/mL and 50–60% degeneration of neurons in brain at 10–100 mg/L dose range [133]. It seems few minerals in their nanoforms are more toxic than their coarser forms. Therefore, safety and toxicity levels of different nanominerals should be widely evaluated before recommending them at an optimum dose for their use in poultry.

15. Other nanoparticles in poultry nutrition

Many other nanomaterials have been used in poultry nutrition. For instance, turmeric extract enclosed in a nanocapsule improved meat quality traits without affecting performance as a feed additive for regular broiler feed and 0.2% turmeric nanocapsule was the optimum level to obtain the best feed efficiency, whereas 0.4% of the nanocapsule decreased liver cholesterol and subcutaneous fat, but concentration at >0.4% reduced growth in birds [134]. Clay minerals of nano-suspensions added at 1 to 2% of the suspension in drinking water in broiler chickens were found to improve feed conversion ratio, body weight gain and antibody titer against Newcastle disease, infectious bronchitis and bursal disease [135]. There is huge potential of functionalizing many nanoparticles for application in poultry nutrition and feeding [32].

16. Conclusions

Minerals are obligatory for maintaining the higher productivity of poultry and a better bioavailable source at lower cost is the prime priority. In this context, nanominerals have produced encouraging responses in most of the studies. Though inconsistent, nano form of Zn, Se, Cu, Ca, and P mostly produced improved responses in poultry reflected by their performances such as body weight gain, feed efficiency, immune responses, egg production, egg quality traits, bone quality parameters, retention of minerals, and enzyme level. The inconsistent performance may be attributed to the level of minerals present in feeds itself and also the varied doses used depending on the hypothesis of the researchers. Many cases have given similar responses even by reducing the dose to half of the conventional inorganic doses, which is suggestive of better bioavailability. Apart from the biological effects in birds, the nanominerals are found to reduce the environmental excretion of the minerals by virtue of its better bioavailability and also reducing the dose of supplementation as well. Considering all the aspects, this can be suggested that use of nanominerals in poultry ration can be considered as an environmental protective strategy to augment poultry productivity. However, further studies with more replicates should be advocated along with long term exposure to validate and unveil more aspects of nanominerals.

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Chapter 6

Effect of Environmental Temperature on Water Intake in Poultry

Ochuko Orakpoghenor, Ngozi Ejum Ogbuagu and Lawal Sa'Idu

Abstract

Water is an essential nutrient in animal nutrition, makes about 70 to 80% of lean body mass and plays important roles in poultry metabolism and thermal homeostasis. Water provided as drinking water constitutes the largest proportion of water available to poultry followed by metabolic water and that available in feed. The intake of water by birds varies depending on the age, environmental temperature, relative humidity, certain diet constituents, type of drinkers used and rate of growth. An increase in environmental temperature tends to cause an increase in water intake while decrease in environmental temperature causes decrease in water intake. Hence, in addition to its nutritional role, water is more important for thermoregulation in chickens especially under hot conditions.

Keywords: water, poultry, homeostasis, temperature, thermoregulation

1. Introduction

Water is the most abundant and widely distributed chemical compound in the world [1]. Though water in the natural state is one of the purest compounds known, there is difficulty finding a freshwater source that has not been altered by man [2, 3]. The use of drinking water with high physical, chemical and microbiological qualities is vital in poultry production as one single water source serves several birds. Hence, any problem in water quality would affect a great number of birds [4–6]. Birds consume approximately 1.6–2.0 times as much water as feed on a weight basis [7]; therefore, any deviation in water quality could have more pronounced effect on poultry health and production than feed [7, 8].

Water is an essential nutrient in animal nutrition and plays roles in poultry metabolism including thermal homeostasis [9], food digestion and absorption, nutrients transport, and waste products elimination from the body [10]. It also makes up 70 to 80% of lean body mass by weight in birds [11–13].

2. Sources of water in poultry nutrition

2.1 Drinking water

Drinking water constitutes the greatest source of water to poultry and it is made available in drinkers [1]. It is of great concern to poultry producers due to its great variability in quality and potential for contamination [5]. It could be from tap, stored in rooftop tanks, or underground (well) water [7]. The physico-chemical parameters established as indicators of water quality include taste, color, odor, pH, electrical conductivity (EC), hardness, alkalinity, salinity, and presence of cations and anions [14]. High-quality drinking water has been defined as water that contains inclusions, which promote vitality and lack inclusions causing morbidity and mortality [15, 16]. Because no water in nature is 100% pure, different water sources will have varying degree of water inclusions, which directly or indirectly affect poultry performance and welfare [17].

2.2 Water in feed

This is the water available in the feed. However, feeding of wet mashes to poultry has not been recommended for use in large scale commercial poultry production, on the basis that it does not offer any nutritional advantage and is difficult to apply [18].

2.3 Metabolic water

Metabolic water refers to water created inside a living organism through their metabolism, by oxidizing energy-containing substances in their feed [19].

Production stage	Age/rate of production	Liters of water/1000 birds
	4 weeks	100
Layer pullet	12 weeks	160
	18 weeks	200
Laying hens	50% production	220
	90% production	270

Table 1.
Typical daily water consumption for layers at 21°C [22].

Age (weeks)	Liters of water/1000 birds
1	65
2	120
3	180
4	245
5	290
6	330
7	355
8	370

Table 2.
Typical daily water consumption for broilers at 21°C (liters per 1000 mixed sex birds) [22].

Age (weeks)	Galons of water/1000 birds	
	Toms	Hens
1	10	10
2	20	15
3	30	20
4	40	30
5	55	40
6	75	60
7	100	80
8	125	100
9	150	115
10	170	130
11	190	140
12	210	150
13	215	160
14	220	170
15	220	
16	220	

Table 3.
Typical daily water consumption (galons) for turkeys at 21°C (liters per 1000 mixed sex birds) [23].

Birds excrete uric acid and can have a net gain of water from the metabolism of protein [20]. Migratory birds have been reported to rely exclusively on metabolic water production while making non-stop flights [21].

Tables 1 and **2** [22] and **Table 3** [23] provided data on typical water consumption levels for layers, broilers and turkeys, respectively, at 21°C.

In quail chicks, the water requirement has been reported to change with increase in age, quantity and quality of feed dry matter. This has been documented to be 3:1–4:2 g/g body weight at 12–29 days of age following stabilization at around 2 g/g body weight. The water feed ratio for the above period are 2:0–2:3 respectively [24, 25].

3. Factors affecting water intake

Birds, like most mammals, are considered to be homeothermic, and they maintain their deep body temperature at about the same level over a wide range of ambient temperatures [26]. Water intake will vary depending on age, environmental temperature, relative humidity, certain diet constituents, type of drinkers used and rate of growth [27]. Water consumption can be limited if the water is too hot or is contaminated with excess minerals [28, 29]. Water and feed consumption rates are interdependent, so reduced water intake can also lead to reduced feed intake [30].

3.1 Effects of environmental temperature on water intake

It has been reported that the thermoneutral temperature for broiler chicks up to 7 days of age ranges between 28 and 35°C, and that temperatures higher than these may induce hyperthermia and dehydration, leading to a lower feed consumption and delayed growth [31–33]. On the other hand, a lower environmental temperature induces hypothermia and may lead to pulmonary hypertension in broilers.

Chicks exposed to low ambient temperature (20°C) had lower water intake than chicks brooded at high environmental temperature (35°C) as reported by Moraes et al. [34]. These were related to the heat conserving behavior of these birds, since at low ambient temperature (20°C) they clustered to maintain optimal heat thus reducing the frequency to the feeder and drinker. Similar decrease in water intake has been reported in birds above 2 weeks of age but with increased feed intake and metabolism patterns [35]. Since poultry are homeotherms that can live comfortably only in a relatively narrow zone of thermoneutrality [36], they are forced to increase feed consumption under low temperatures in order to balance their body temperatures [37].

Broilers subjected to acute heat stress have been reported to show higher water intake. The water intake increases in order to maintain thermoregulatory balance [38], as heat stress induces high water loss through the respiratory. This acts as a means to achieve efficient thermoregulation through evaporative cooling. In critical heat stress situations, water loss may cause marked changes in the thermoregulatory balance of poultry [39] and this may result in death.

4. Conclusion

Increase in environmental temperature tends to cause an increase in water intake while decrease in environmental temperature causes decrease in water intake. Hence, in addition to its nutritional role, water is more important for thermoregulation in poultry especially during hot conditions.

Conflict of interest

The authors declare no conflict of interest.

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Section 2

Nutrition and Health

Chapter 7

Gut Health and Immunity in Improving Poultry Production

Naga Raja Kumari Kallam and Veerasamy Sejian

Abstract

A healthy gastro intestinal system is important for poultry to achieve its maximum production potential. This paper aims gut health and immunity to improve production in the poultry sector. Genetics, Nutrition and Bio security ate the factors influences the production. Gut consisting of various pH and micro biota throughout is an advantageous feature to prevent infections. Various components like Goblet cells, paneth cells, endocrine cells and absorptive enterocytes, tight junctions, GALT and Mucus play a major role in gut health. Balanced diet with optimum carbohydrates, proteins, amino acids, minerals, vitamins, enzymes, organic acids and good management practices are important for improving production. Alteration in supplementation essential amino acids, Zn, Vit E, Se … viz. are needed according to changes in environment and production state of the bird to develop good immunity. Stress free environment with fine hormonal balance are imperative for maximum output. Exploration of genes involved in resistant to food borne pathogens and research towards bio markers for gut health is the need of the hour. In can be concluded that good gut health and immunity play a key role in production. These can be achieved y maintaining birds with optimum nutrients and stress free environment.

Keywords: gut health, immunity, micro biota, poultry, production

1. Introduction

The emerging global agrarian crisis associated with the growing human population has signified the role of livestock sector in catering their nutritional demands. Among the livestock species, poultry sector has emerged as the fastest growing enterprise over the years with their significant contribution to the total animal protein production as well as consumption [1, 2]. Accompanying this growth, the poultry industry is faced with an enormous challenge to maintain the health and well-being of the birds.

1.1 Global significance of poultry production

Remarks of [3] indicate predicted human population by 2050 is 2097 millions means expecting addition of 877 million to the existing population of 1220 millions.

As of now the available eggs are 58no's, and meat is of 2.2 kg/capita/annum [4]. As per [4] report growth rate of poultry industry is 7.52% and 8.51% in broiler and layer respectively to cater the needs of animal protein demand. Conversely, the projected growth and production output was mainly depends on availability of quality feed ingredients, over and above the emerging and reemergin diseases.

The growth and development of poultry industry is crucial in sustaining the supply of safe, nutritious and good quality protein sources to balance the diet composition of humans. Productive performance of poultry had influenced by the host factors like: genetic background (bird species, type of bird, breed and sex), gut development and maturation (immune system, gut morphology and micro biota acquisition) and stress (environmental factors, type/form of feed, poor management, health interventions, litter management, nutrition). Gut health is a major concern and more complex with more than 640 different species of microbiota, over 20 hormones which digest and absorb the majority of the nutrients accounts 20% of energy utilisation [5]. It infers that factors fatal to gut certainly influences the nutrient intake as well as compromised output.

A healthy gastro intestinal tract (GIT) will be able to efficiently carry out activities viz. protection against insults (infectious and non-infectious), transport of ingested feed and digesta through the entire track, digestion, absorption process, hosting microbiota and excretion of undigested feed. Whereas, it may not be possible with a compromised GIT due to various deviations. Factors like nutrients/feed; microbiota and environment/stress will influence the functionality, integrity and health of gut.

1.2 Factors to be considered for good gut health

1. Environment factors: improper ventilation and temperature causes poor development and functioning of intestinal epithelium both in catabolism and absorption.

2. Bio security: poor management and improper bio security leads to the domination of pathogenic bacteria in the gut.

3. Nutrition: sudden change in feed, feed composition will affect the balance of gut microbes. Mycotoxins in the feed also damage the gut tissues.

4. Challenging situations like vaccination and disease out breaks.

5. Early Nutrition/brooding: making the feed available immediately after the hatch fastens the development of gut. Sub optimal management impairs the gut development and immune system.

Here in this chapter is an insight into the role of gut health and integrity, through their roles, in modulating bird's ability to be resilient towards the infections on immunity and production.

2. Importance of gut health

The immune and non-immune cells in the gut interact and help to provide intestinal protection, tolerance and homeostasis. In general, these components work in coordination with each other to maintain homeostasis as well as to prevent diseases, in turn maximise the utilisation of acquisition of dietary nutrients and further leads to improvement in production.

2.1 Gut health

Gut health influences nutrient assimilation, intestinal barrier and integrity, immune response and efficiency, inflammatory balance, susceptibility to enteric pathogens. The livestock/poultry utilises several non –specific ways to maintain their health status. Lower pH of the gut and cilia in the respiratory tract is one among the additional barriers in this scenario to counter the pathogenic challenges.

2.2 Components of gut and functions

Gut consists of a single layer of epithelial cells and the underlying lamina propria, along with the immune cells and muscularis layers (**Figure 1**). Epithelial cells are in the top most place covered with mucin, include goblet cells, Paneth cells, entero endocrine cells.

2.2.1 Goblet cells and mucin

Mucous layers covering the epithelia secreted by Goblet cells, and act as a protective barrier, defending the underlying epithelium from damage and infection by pathogenic bacteria and acts as a substrate and fixing medium for communal bacteria [6]. Improper coverage of epithelial by mucous harbours more invasive to pathogenic microbes [7, 8]. Proper nutrition is important for good mucus production and gut immunity to prevent colonisation of infectious microbes.

2.2.2 Paneth cells

It plays an important immune defence role in the intestine, particularly relating to production of host defence peptides (HDPs), repair of intestinal epithelium especially during damage/inflammation. Paneth cells are majorly located in small intestine crypts. Along with goblets cells, entero ednocrines these will form tight junction. Tight junctions are nothing but multiprotein complexes, form a continuous intercellular barrier between epithelial cells and regular para cellular

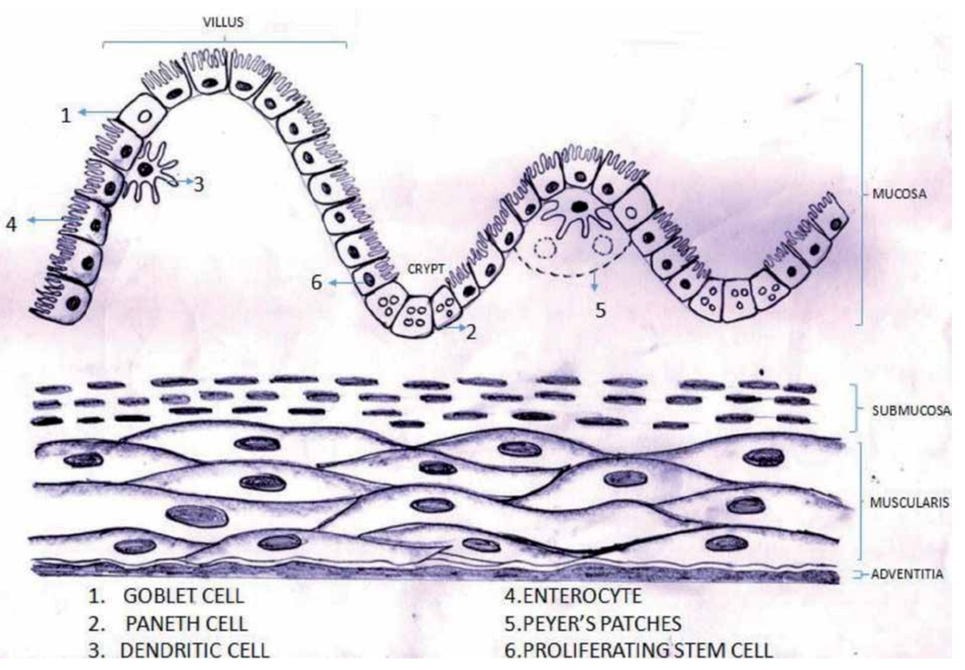

Figure 1.
Cross section of small intestine.

permeability. More number of Paneth cells are presents at small intestine crypts i.e. Crypts of Lienerkuhn. These consist of ER, Golgi network and large granules produce HDPs viz. α-defensins/cryptdins, secretory phospholipase A_2 and lysozyme.

Antimicrobial peptides or immune modulators released from the Paneth cells protect the host from enteric pathogens. The glycosidase secreted from the paneth cells named Lysozyme, which hydrolyses peptidoglycan and is one of the major components of the bacterial cell wall. Lysozyme also observed in gastric and small intestinal secretions, as well as in the granules of macrophages and neutrophils and the G_2-type, which is identified in the small intestine, liver, and kidney [9]. In the gastrointestinal tract, lysozyme C is expressed in the gastric and pyloric glands, duodenal Brunner's glands, and in the Paneth cells.

2.2.3 Enteroendocrine cells

Even though they are less in number, compared to other intestinal epithelial cell population (about 1%). In response to chemical/mechanical stimuli these cells secretes a variety of hormones viz. GLP-1, GLP-2, PYY, CCK and serotonin. These play a very important role in birds while regulating hormone secretion, gastrointestinal enzymatic activity, and feeding behaviour.

2.2.4 Lamina propria

Lamina propria is a loose connective tissue. Consist of dendritic cells, macro phages, heterophills, natural killer cells immune cells like T and B cells and intra epithelial lymphocytes.

2.2.5 Microbiota

Microbiota forms a protective layer by attaching to the epithelial walls of enterocyte and minimise the options for the colonisation of pathogenic bacteria by competitive exclusion. Microbiota plays an important role in production of Short chain fatty acids, organic acids, vitamins (B group and K), bacteriocins (Antimicrobial property), lower triglycerides finally induce non pathogenic responses which give both nutrition and immunity to the bird [10, 11]. Incontrast some of the pathogenic bacteria like Salmonella and camphylobacter which may circulated to humans while consumption may act as a pool for antibiotic resistance and transmission which is having more public health concern [12].

The chicken gut microbiota (**Figure 2**) includes hundreds of bacterial species dominated at the phylum level by Firmicutes (*Lactobacillus, Clostridium, Ruminoccocus, Turicibacter),* Bacteroidetes *(Flavibacterium, Fusobacterium*), Proteobacteria (*Enterobacter*) and Actinobacteria(*Bifidobacterium*). The microbial communities differ throughout the chickens GIT with particular microbial profiles have been detected in crop, gizzard, ileum, cecum and colon of chickens.

Functional output of microbiota includes production of SCFAs [13–15] added metabolic potential and competitive exclusion of pathogens by decreasing the pH. Lactic acid bacteria (LAB) favour renewal and barrier function of the gastrointestinal epithelium by SCFAs production [16].

Fatty acids produced from microbiota plays an important role. Among the SCFAs acetate is produced more followed by propionate and butyrate. Even though less amount of butyric acid is produced it acts as a source of energy for colonic epithelia and helps in maintaining homeostasis at colonocytes and further helps in good morphometry. Functions like enterocyte growth and proliferation, mucin production, intestinal blood flow, intestinal immune response are influenced by the SCFAs.

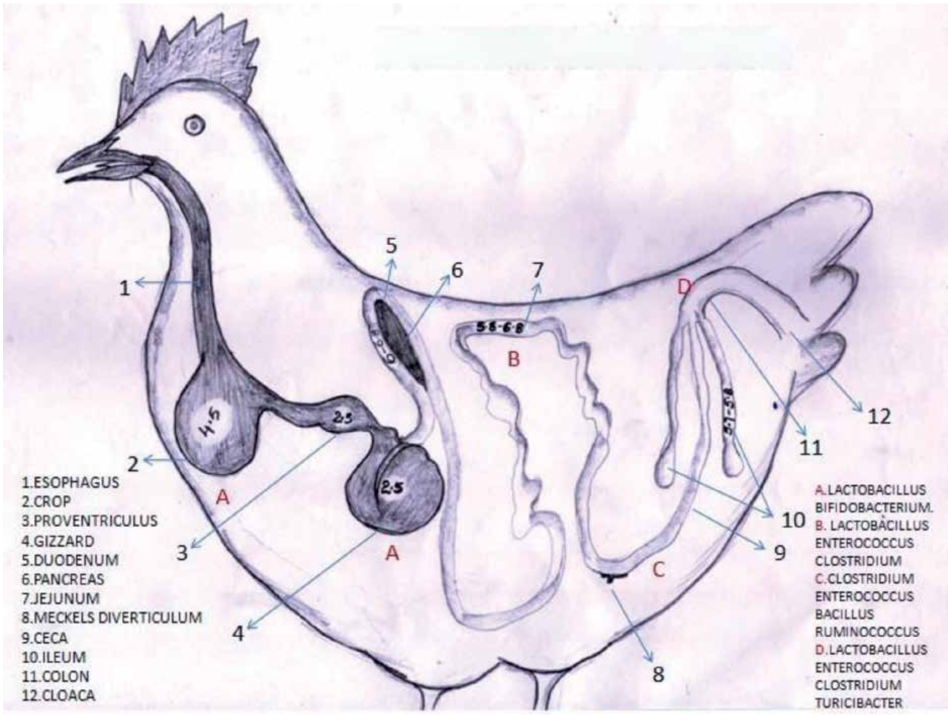

Figure 2.
Microbiota in gut of fowl.

An imbalanced microbes in the gut is termed as Dysbiosis, this imbalance is majorly due to stress, change in nutrition and infections which alters immune system. It causes a sequential reaction in the GIT, including reduced intestinal barrier function (e.g., thinning of intestinal wall) and poor nutrient digestibility, and therefore, increasing the risk of bacterial translocation and inflammatory responses [17]. The severity of dysbiosis varies depending on situation but it is generally characterised by loose and foamy caecal droppings. Post-mortem examination of affected birds reveals thinning of the gut wall along with gassy and watery gut contents.

Proper bacterial balance i.e. at least 85% of total bacteria should be good bacteria is vital for the host, and the impact on gut health often comes from microbial imbalance in the gut of chicken [18].

In micobiota phylum Bacteroidetes (i.e., *Bacteroides fragilis*) associated with production of IL-17 producing T cells [19]. Lactobacillus group of commensal bacteria stimulate intestinal immune system and increase the resistance to diseases, as part of that brings about release a low molecular weight peptides [20].

2.3 Activities in gut

Anti microbial peptides (AMPs) and Ig A are secreted in the gut by the epithelial cells [12]. Mucus layer protects the epithelium and acts as a first line of defence against infection. Small intestine goblet cells in chicks starts functioning in the late embryonic and post hatch period and can be observed by 17th day of incubation. At that time the produced mucin is of acidic in nature. Whereas, until day 7 after post hatch the same goblet cells produce both acidic and neutral mucin. Later age onwards expansion of goblet cell number towards ileal axis get initiated [19].

3. Factors influencing gut health

3.1 Nutrients in relation to gut development & Health

In diet certain nutrients play a crucial role in metabolism, absorption as well as participate in maintain gut health and homeostasis. In diet ingredient quantity, quality, form, type, processing method, additive types..etc. play a dynamic role in maintain gut microbiota and integrity [20]. Some of the reports regarding application of nutrients more than recommendation and its impact on gut health was presented in **Table 1**.

3.1.1 Carbohydrates

Production of molecules involved in defecen mechanism like monocytes and heterophils, Ig, nitric oxide, lysozome, communication moluclues (eicosanoids, cytokines, and clonal proliferation of antigen-driven lymphocytes) are depending on availability of energy, amino acids, enzyme co factors etc. [20].

Energy from carbohydrate source is the best choice since immunological stress is known to impair triglyceride clearance from the blood, thus decreasing fat utilisation. Ingredients of plant origin contain considerable amounts of fibre (non-starch polysaccharides, NSP, plus lignin), with the majority being insoluble which has various roles in improving gut health, enhancing nutrient digestion and modulating the behaviour of animals. It is postulated that monogastric animals have a 'fibre requirement' because their gut development requires physical stimulation by hard, solid particles of feed [34].

3.1.2 Proteins and amino acids

Using dietary protein intake as an example, at elevated temperatures, digestion and absorption are altered, favouring protein catabolism, and subsequently a reduction in protein synthesis and deposition [35]. Kumari et al., [36] reported that low protein diets (17 vs.15.58 & 13.4%) for layers supplemented with ideal amino acid lysine @ 0.70% at high temperature (32°C) from 25 to 36 weeks of age is essential for better performance on par with 17% protein in diet. However, Temim et al. [37] reported that high protein diets (28 and 33%) improved the performance when compared to low protein diets (20%) in broiler chicks. Burkholder et al. [38] noticed that stress conditions either due to high temperatures or due to fasting for more than 24 hrs, changes in microbial population and increased attachment or colonisation of pathogenic bacteria like salmonella.

Role of some amino acids (Glutamine, Arginine, Threonine and Cysteine) on the integrity, growth, and development of the intestinal epithelium, gene expression, cell signalling, antioxidative responses, and their associated immune functions have been investigated by [39–42].

Digestion and absorption are influenced by gut health and villi length especially infections which damages the epithelia during such situation supplementation of amino acids favours the production of SCFAs which improves the immunity [43, 44].

3.1.3 Minerals

Research findings inferred that the role of trace minerals in maintaining the intestinal health supports anti oxidant system, balancing micro biota and repair. Shanon and Hill [45] reported importance of copper in growth and mechanism of gut, high concentration of Zn prevents proliferation of pathogenic (clsotridum and E.coli) bacteria.

Strain/type application	Biological activity	Reference
Probiotics		
L.reuteri, L.gallinarum, L .brevis, L .salivaris	Lactobacillus and bifido bacter population is increased and E.coli counts are decreased in caeca.	Mookiah et al. [21]
L. acidophilus	Induced T-helper-1 cytokines in caecal tonsil cells	Brisbin et al. [22]
L. salivarius	Induced anti-inflammatory responses (interleukin [IL]-10 and transforming growth factor [TGF]-b) in caecal tonsil cells	
L.plantarum,L.bulgaricus, L.acidophilus,L.rhamnosus, B.bifidum,S.thermophilus,E. faecium, A. oryzae and C. pintolopessi	Increased antibody titre against Newcastle disease (ND). Increased the geometric means haemagglutination inhibition (HI) titres of birds	Khan et al. [23]
Saccharomyces boulardii	Increase in intestinal villus height and crypt depth in the jejunum and ileum and goblet cell number.	Naga Raja Kumari and Susmita [24]
Prebiotics		
FOS	Provided nutrients for the growth of beneficial bacteria in the gut	Alloui et al. [25]
Inulin	Increased bifidobacterium counts and decreased *E. coli* counts in caecal contents	Nabizadeh [26]
GOS	Increased Bifidobacterium spp. and decreased Campylobacter spp. in the faecal samples	Baffoni et al., [27]
IMO	Increased the caecal populations of lactobacilli and bifidobacteria and decreased the caecal E. coli	Mookiah et al. [21]
Fibregum and Raftifeed-IPE	Increased serum concentration of IgA and IgM, and enhanced systemic immune capacity in chickens	Vidanarachchi et al. [28]
Prebiotic-based MOS and b-glucan	Increased the relative weight of spleen, decreased the heterophil-to-lymphocyte ratio and increased antibody titres against S. enteritidis	Sadeghi et al. [29]
Symbiotics		
Commercial synbiotics (BiominImbo)	Increased the LAB population and reduced E. coli and total coliform populations in the intestine	Amerah et al. [30]
Bifidobacterium-based symbiotic product	Reduced C. jejuni concentration in poultry faeces	Baffoni et al., [27]

Strain/type application	Biological activity	Reference
Synbiotic 11 Lactobacillus strains plus IMO	Increased the caecal populations of lactobacilli and bifidobacteria and decreased the caecal E. coli	Mookiah et al. [21]
Exo genous enzymes		
Carbohydrase	Increased the proportion of lactic and organic acids, reduced ammonia production, and increased VFA concentration through hydrolysis fragmentation of NSP and supporting growth of beneficial bacteria.	Adeola and Cowieson [31]
Xylanase	Minimise the counts of Salmonella	Amerah et al. [30]
Exogenous enzymes	change in the gut microbial populations increased immunity	Bedford and Cowieson [32]
Xylanase	Reduced crypt depth of jejunum	Yang et al. [33]

Table 1.
Results of some of the works in application of additives in poultry diets.

Whereas, Baxter et al. [46] inferred that chelated minerals and EAA at optimum concentration in diet has great influence on gut health especially during heat stress.

Zn in the form of ZnO protects the intsetianal cell from E.Coli infestation, inhibits adhesion and internalisation of bacteria, prevents the commotion of barrier integrity, and modulates cytokine gene expression [47].

Several elements in diet have been shown to have a negative effect on the immunological response in the bird when they are deficient in the diet. Several studies revealed that adequate levels of Zn supplementation (between 50 and 70 mg/kg) in poultry diet have been shown to minimise the impact of oxidative damage in the intestine of broilers under stress [48, 49]. Zinc is especially important in wound healing, thymic function and proliferation of lymphocytes. Growth depression usually observed due to catabolic response during nutritional stress induced immune stimulation.

3.1.4 Vitamins

Vitamin E appears to be an immune system "booster" and by inhibiting the synthesis of prostaglandins [50].

Vitamin A deficiency [51, 52] and excess [52, 53] have been shown to depress immune responses in chicks. Most research suggests that vitamin A deficiency is associated with reduced cellular immune responses whereas; vitamin A excess impairs antibody responses.

To reduce stress in the form of vaccination/handling/environment boosting the diet with B vitamins, fat-soluble vitamins (A, D, and E), and electrolytes in drinking water are helpful in antibody production and also to reduce mortality from the stress [54].

3.1.5 Probiotics

Feed microbials are used to colonies in the intestine with microbes of desirable attributes that can promote competitive exclusion and/or promote beneficial gut barrier and immune function. Choice of in feed eubiotic additives in diets have positive influence on intestinal microila community and to improve the birds immune system [55–57].

Commonly used probiotics species at poultry industry are LAB, i.e., *Lactobacillus bulgaricus, L.acidophilus, L.casei, L.helveticus, L.lactis, L.salivarius, L.plantarum, Streptococcus thermophilus, Enterococcus faecium, E. faecalis, Bifidobacterium spp.,* [55, 58].

3.1.6 Prebiotics

Prebiotics are non digestible feed ingredients acts as a substrates for microbes, can helps in shaping the gut microbiome and, in turn, developing the immune capabilities [57, 59]. Prebiotics provide nutrients for endogenous favourable bacteria like bifido acteris and LAB and improves host microbiota balance [57]. Oligosaccharides (inulin, fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS), soya-oligosaccharides (SOS), xylo-oligosaccharides (XOS)) pyrodextrins, isomaltooligosaccharides (IMO) and lactulose..etc. [25, 59, 60] are commonly used prebiotice in poultry.

3.1.7 Synbiotics

A combination of probiotic(s) and prebiotic(s), which attempts in providing favourable microorganism(s) along with a nutrient source.

3.1.8 Exogenous enzymes

Alter the availability of nutrients from the substrates and increase the digesta viscosity. Poultry rations are mainly based on ingredients of palnt origin with more phytate phosphorus, to make use of P from the source phytase enzyme are normally used, apart from this proteases, NSPases are very common [61]. Various exogenous enzymes including b-glucanase, xylanase, amylase, a-galactosidase, protease, lipase, phytase, etc. have been supplemented in poultry diets for decades [31, 32].

3.1.9 Phytobiotics

Phytobiotics are the compounds (terpenoids, phenolics, glycosides and alkaloids etc.) derived from the plants, which posses antimicrobial and some functional properties [59, 62]. Some of the phytobitics enhance the growth of useful bacteria like LAB in gut, improves immunity, protects intestinal cells by altering the membrane permeability [63, 64].

3.1.10 Organic acids

Butyric acid, Lactic, acetic, tannic, fumaric, propionic, caprylic acids, etc. comes under this category, which had antimicrobial properties, enhance gut structure and alter pathogen gene expression and enhance the performance of birds [65, 66].

3.1.11 Polyunsaturated fatty acids

Fish oil and corn oil are the main source of n-3 fatty acids and n-6 fatty acids, respectively in poultry diets and improves the body's function and immunity [56].

3.1.12 Mycotoxin mitigation

This limits intestinal damage and suppression of immune responses for application in the gut health arena.

3.2 Immune system in relation to gut health

Genetically superior birds are in immunologically stress full condition which creating pressure in the producers due to ban of antibiotic growth promoters. This creates tremendous pressure on immune system of the birds by various vaccination schedules.

Feed deprivation at early stage of life delays the development of mucosallayers and process of mucin synthesis in chicks [67].

3.2.1 Position of lymphoid tissues associated with mucosa and spread in different organs: mainly GALT

(Gut associated Lymphoid Tissue): Meckels diverticulum, Payers patches (Intestinal wall), Oesophagus –proventriculus junction, caecal tonsils. Additionally, gallbladder, liver, pancreas, kidney and oviduct are also having some role in protection and immune competence in birds [67].

Gut is the key immunoclogical organ comprises of myeloid and lymphoid cells [68] with its associated structures it forms a site for production of many immune cell types that needed for initiate and mediate immunity.

Immune system in gut depends on microbiota and maturation of the system eliciting by antigen-specific responses mainly influenced by dendritic cells [69]. The non invasive microbes initiates the production of IgA, which controls the host commensal interaction by both impacting commensal gene expression in the lumen and preventing adhesion of commensal bacteria to the epithelial surfaces [70].

3.2.2 Interrelationship between nutrition and immunity

Poultry encounter numerous stressors during their lives. Alterations in feed intake, poor FCR, varied nutrient metabolism, suppressed immune system are the consequences due to stress [71]. To overcome stress birds defence system prioritise the nutrient utilisation during challenge period [72].

Birds immune system get affected by nutrition in several ways [73] like.

a. Development of lymphoid tissues.

b. Mucus Secretion by goblet cells.

c. Production of immunologically active substances.

d. Cellular proliferation and activation

e. Intracellular killing of pathogens.

4. Stress

Another important factor influences the suppression of immunity as well as production is stress [48]. It may be due to environment (other than comfort zone), diet, vaccination- and medication, microflora imbalance, as well as a result of pathogen or parasitic load [74]. Disruption of gut function may occur and imbalance between production and elimination of ROS is a common phenomenon during stress [48]. Excess ROS in intestine causes destruction of PUFA in the cell membrane and leads to production of peroxides (malondialdehyde) which ruin the intestinal integrity [40]. A compromised epithelium creates a good opportunity for opportunistic pathogens, in addition to this dietary deficiencies in certain nutrients can increase the stress-induced susceptibility of poultry to oxidative stress [48, 49, 75, 76]. The outcome of this is an economic loss to the producer. Improper management makes the poultry exposure to disease causing agents, stress, and Meagre immunity, birds prone to less production or even death [76]. Increased feed efficiency and gut integrity was noticed [77] during in vitro experiments by addition of *Lactobacillus* species which produced bacteriocin Reuterin that might inhibit the growth of *Salmonella, Shigella, Clostridium* and *Listeria*.

5. Endocrine regulation of gut health in poultry

Intestinal microbiota structure and function plays a crucial role in health and production of poultry. The micro biota modulates the intestinal homeostasis, integrity and are essential for good out put [78].

Two types of signals produced by the gastrointestinal tract which influence the feeding behaviour and feed intake, which were in turn influenced by ghrelin, CCK, GLP-1,GLP-2 and PYY and these are released from the intestines and transmit satiety signals to the brain after food intake, resulting in the suppression of appetite [78].

6. Genes that govern gut health and immunity

Genes present in the body will regulate all the activities in the body including gut, but in gut, genes do not have any role without the microbiota [78]. It is considered that microbiota will regulate the activity of genes in the gut. The microbiota present in the gut mainly defines the gut condition whether it is in good condition or not [79].

The main target of Nutrients to alter the activity of genes and results in more activation of good genes and suppresses the activity of bad ones. Through nutrigenomics careful selection of nutrients for fine-tuning genes and DNA present in every cell and every tissue of an animal is possible stated in the review [80]. In **Table 2** results of some the researches were presented regarding influence of nutrients on gene expression.

Jiang et al. [86] reported variations in expression pattern of hepatic genes apolipoprotein A-I (ApoA-I) and apolipoprotein B (Apo B) with varying amount of nicotinic acid in feed indicates the lipid metbalosim. Addition of prebiotics likes mannan oligosaccharides (MOS) to broiler diets increases the expression of mucin, and down-regulates selected genes involved in cell turnover and proliferation [78]. Delay in feeding immediately after hatch alters the hepatic gene expression [87]. Nutrigenomics provides a way to identify precisely which nutrient or nutrient combinations that is optimal to elicit maximum benefits [79].

	Gene expression	Reference
Immunomodulators like Corticosterone, ascorbic acid and 1,3–1,6 β-glucans.	Cytokine gene expression (ILreceptors 4 and 15) in spleen-1β, IL-2,toll-like.	Kumar et al. [81]
MOS	Expression of 77 protein synthesis gene, including superoxide dismutase 1, lumican, β 2-microglobin, apolipoprotein A-1, fibronectin 1 etc.	Xiao et al. [82]
High and low nutrients (HN and LN)	The gene expressions of Rheb, TOR, S6K1 and 4E-BP1 in muscle were the highest in the WYFC fed with LN diets.	Wang et al. [83]
Lead	Down regulation of all sugar, peptide and amino acid transporters. Up regulation of stress related genes.	Ebrahimi et al. [84]
Low protein (13.46% CP and 0.65% lysine at 66% threonine) diets at peak production in layers	Up regulation of MUC2 expression	Kumari et al. [85]

Table 2.
Gene expression related to nutrition in chicken.

Nutragenomics is an emerging science which would reveals the diseases induced by nutrient availability or alteration in diets [88]. For sustainable poultry production there is an emerging need to trace the relationship between economic traits and dietary regimen [57].

Among the different diseases occurring in poultry, those caused by the genus Salmonella is the most common, causing serious economic losses to the poultry industry in terms of mortality, reduced growth and loss of egg production [89].

Some of the works [29] inferred that MHC-B haplotypes reactions were different towards genetic resistance against salmonellosis. Resistance to salmonella was linked to ILs, IFNγ, TLRs, iNO and apoptosis genes, and expression of IL-2, IL-6, IL-8 and IFNγ genes was higher, microsatellite analysis inferred [90] that MHC-1class was linked to colonisation to salmonella. Ocak et al. [91] noticed salmonella at gensus level by PCR-RV, and ST at serovar level.

7. Bio markers

Determination of biomarkers to know the intestinal health is important breakthrough. Inflammation is the first sign of infection or injury to the system [92].

Baxter et al., [46] reported serum citrulline and IFN-È, cloacal IgA, are the potential biomarkers to identify the inflammation in intestine due to dysbacteriosis, and impaired gut permeability.

Alterations in gene expression of claudin-1 and occludin indicate mitochondrial respiration and ATP production variations which indicate the state of intestinal permeability at tight junctions and celluar energy status [89].

Whereas oxidative stress is measured [93] by superoxide dismutase enzyme activity Thiobarbituric acid reactive substances produces during peroxidation and damage of the cells, break down of nitrite and nitrate produces nitricoxide which can be noted by griess assay to identify the concentration of nitric oxide in the cell [74].Pathogens like coccidian directly damages the epithlieal cells and loss of villous epithelia, results in decreased absorption [94]. Simple techniques like measuring the villi length, crypt depth and ratio are helpful standards to know the gut health. Villus height at duodenum, jejunum and ileum are 1400,900 and 700 mm and crypt depth of 190,170,160 mm with villus height to crypt depth ratio were 8, 6 and 5 were reported by Verdal et al. [95] in broilers at 23 days of age.

8. Conclusion

The gut represents a continuously evolving ecosystem where a dynamic interaction between host immune, neuroendocrine and entero-endocrine cells. The gut micro biota of the birds has influence on normal physiological development and homeostasis. Ban on AGPs make the evaluation of precision feeding as well as availability of various additives, phytobiotics, nutraceuitical…etc. are forcing the nutritionist to formulate the least cost rations for better production. Change in dietary regimen to boost immune system, minimise stress, good management and bio security practices are needed. Identification of genes/bio markers that regulates health and immune system is essential. Relationship among economic traits, dietary regimen and gene expression are the gaps. This review reveals that nutrition, immunity and stress are factors that influences the gut integrity in birds and production output.

9. Future research

- Introduction of new technologies that unveil the underlying transcriptional and other molecular mechanisms for disease resistance in chicken.

- Technologies that aid in identification of disease resistant genes include next generation sequencing, microarray analysis, RNA sequencing and high density SNP genotyping.

- Exploring of genes related to degradation and digestion of complex NSP for better utilisation of alternative feedstuffs to incorporate in large-scale industrial production.

- Development of biomarkers of gut health is for understanding the pathophysiological events which influence the intestinal barrier, its functionality and the ecology of the GIT microbiota.

- Quantification of *Enterobacteriaceae* using Q-PCR or other means may be of use to measure dysbiosis in poultry.

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Chapter 8

Secretory Defense Response in the Bird's Gastro-Intestinal Tract and Nutritional Strategies to Modulate It

Luis-Miguel Gomez-Osorio, Zhengyu Jiang, Qian Zhang, Hui Yan, Ana-Maria Villegas and Todd Applegate

Abstract

The Gastrointestinal tract (GIT) is a very complex environment which converges a lot of players including nutrients, microorganisms, pathogens, cells, and peptides which determine the type of outcome against threats affecting feed efficiency and body weight gain. Traditionally, GIT is examined as a selective barrier which permit or deny the entrance of molecules, but beyond of that, it is a crucial place to produce important proteins for the host which, at least in part, determine the outcome for a threat such as microorganisms, toxins, anti-nutritional factors, among others. During the non-antibiotic promotants time, there is a necessity to understand how this system works and how we can modulate through nutrition, in part to take advantage of this, and support a better immune response and nutrient absorption in challenged poultry environments. The goal of this chapter is to review the different mechanism of immunity in the GIT emphasizing on secretory defense response and the nutritional strategies including fiber and fatty acids to improve it.

Keywords: antibiotic growth promotants, mucins, M cells, paneth cells, goblet cells, host defense peptides, trefoil factors, IgA, dietary fiber short chain fatty acids, medium chain fatty acids

1. Introduction

There is a tremendous interest in the understanding of immune response against pathogens and toxins on the gastrointestinal tract (GIT) of the birds due to in this specialized system, as it harbors 70 to 80 percent of the avian immune cells and molecules [1]. Additionally, there is an overwhelming interest in finding new alternatives to antibiotic growth promoters (AGP) because of regulations and consumer preference in many countries which these strategies are banned or regulated. The mucosal surface of the GIT is covered by a monolayer of columnar epithelial cells. This epithelium represents a vast surface that is vulnerable to foreign immunogens (i.e. food-borne antigens), microbial pathogens and toxins. By being in contact with a large number of potentially harmful substances and infectious organisms, the mucosal surface must provide a means to not only regulate active and passive absorption of macromolecules but also provide as a general and selective defenses

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in part through secretory antibodies and other mucosal defense mechanisms. Consistent with these functions, the epithelial surface of the GIT is lubricated and protected by mucus secretion and by a highly specialized immune system underlying the epithelium which exports immunoglobulins into the intestinal mucosa. Secretory defenses are some of the most important means to protect the intestinal epithelium from enteric pathogens and toxins. Secretory IgA (sIgA) production, Goblet, Paneth, M cells and GALT tissues are the key cells in this defense. The objective of this review is to describe a variety of secretory immune responses against pathogens in GIT and the role of nutrients in immunomodulation.

2. Histology and physiology of the gastro-intestinal tract

Gallus species have villi which decrease in length from 1.5 mm in the duodenum to 0.4–0.6 mm in the ileum and rectum. The number of villi decreases from 1 to 10 days of age, but thereafter remains constant. Genetic selection for growth has altered villi morphology [2]. The villi of broilers are larger than White Leghorns, and show more epithelial cell protrusions from the apical surface of the duodenal villi. However, the villi from both types of chickens consist of a zig-zag arrangement which is thought to slow the passage rate. The intestinal wall contains four layers as including the mucosal, submucosal, muscle tunic, and the serosal layer. The mucosal layer consists of the muscularis mucosa, lamina propria, and epithelium. However, the muscularis mucosa and lamina propria are poorly developed in chickens, possibly because of the absence of a central lacteal. Although Brunner's glands, common to mammals, are absent [3] tubular glands possibly homologous to Brunner's glands, are present in some birds [4]. The epithelium has chief cells, goblet cells, and endocrine cells. The crypts of Lieberkühn are the source of epithelial cells lining the villi. The crypts contain undifferentiated cells, goblet cells, endocrine cells, and lymphocytes. Globular leukocytes and Paneth cells appear near the base of the crypts. The intestine contains extensive innervation from both the sympathetic and parasympathetic nervous system. As described [5], innervation is both cholinergic and adrenergic. Contraction of the rectum appears to be mediated by noncholinergic, non-adrenergic nerves [6, 7].

The mucosa of the GIT is a functional interface between the environment and the internal physiological compartments of the organism. As such, the mucosal and associated cells constitute a dynamic and metabolically active barrier possessing selective permeability [8]. This barrier has multiple functions that involve the digestion, transport and uptake of specific substances and nutrients and exclusion of microorganisms and toxins. The processes of digestion and absorption occur in a micro-environment modified by the intestinal mucosa, its secretions, and the ancillary organs (pancreas, liver). The importance of 'the intestinal barrier' as it relates to gut function and gut health in poultry has been reviewed [9, 10]. Optimal digestive and absorptive functions are essential for growth, development and health of the animal. In addition, the intestine must act as a physical barrier to pathogenic organisms and toxins and play a role in both innate and acquired immunity. The integration of the digestive, absorptive and immune function of the GIT and the genetic regulation of these processes are central to animal production and health.

3. Innate immunity of the GIT

The epithelial cell physical barrier in the GIT represents a vast surface area that is very vulnerable to intraluminal impacts. Continual confrontation by direct contact with foreign substances, the mucosal system is tightly regulated in order to allow selective entry of macromolecules necessary for mucosal defense [11]. The cells and molecules that comprise the innate immune responses encompass both physical and chemical barrier mechanisms. For example, epithelial cells are tightly connected by multi-protein junctional complexes which regulate passage of solutes while providing an obstacle to luminal microbes and the lamina propria. Mucosal epithelial cells also produce non-specific macro-molecules (such as defensins) with antimicrobial action. Inflammatory and anti-viral responses are produced by specific mucosal cell types, which include: dendritic cells (DC), macrophages, and innate lymphoid cells (ILC). Pattern recognition receptors on these cells regulate many of these responses through interaction with microbial ligands [12].

3.1 Mucus and mucins

The intestine is protected by that substance, which forms a tightly adherent layer along the epithelial surface, followed by a more loosely adherent, partially hydrolyzed layer. It is also part of an integral process, and is secreted, forming and "unstirred" water gel layer covering the epithelial surface. This gelatinous molecular "coat" is subjected to continuous erosion by luminal fluid flow and rapid replenishment from epithelial secretion. The dynamics of mucus gel turnover contributes to a complex milieu where digestive events occur, nutrients approach epithelial cells, microbes build ecological niches, exfoliated enterocytes break down and immunological molecules (defensins, IgA, etc.) carry out surveillance. Consequently, the mucin layer, which encompasses all the of these components, constructs a gel-like biological barrier that shields the underlying tissue compartments, and eventually serves as an important component of the innate arm of the host system in the GIT [11]. In the small intestine the mucus layer is penetrable, but the bacteria are kept away from the epithelium by antibacterial mediators. In the large intestine, the inner mucus layer is impenetrable to bacteria whereas the outer mucus layer is expanded and serves as the habitat for bacteria (esp. mucolytic bacteria) [13]. Serving not only as a lubricant but also a protective barrier, the mucus gel layer(s) in the GIT is the largest area and of critical importance to the body both physiologically and nutritionally. Compromised mucin function is associated with many gastro-enteric disorders and nutritional insufficiencies. Particularly, many functional modulations of the GIT are closely related to expressional, structural, and physiological alterations of mucus and its major components [14].

The protective functions of mucus are attributable to mucus glycoproteins, the major macromolecules present in the mucus gel. Mucus glycoproteins, now widely known as mucins, are defined as a class of high-molecular-weight proteins that are heavily glycosylated with complex oligosaccharide chains [15]. The molecular weight of mucins has been estimated from early studies of ~1000 kDa with attached carbohydrates accounting for 80% of the mass [16].

According to cellular localization and distribution, mucins are broadly classified into secretory and membrane-associated proteins [17]. Structurally mucins are comprised of a linear protein backbone in the center and a large number of carbohydrate chains attached around it. The carbohydrate components, usually heterosaccharides, are bound covalently to the peptide chains and terminated with sialic acid (sialoglycoproteins) or with both sialic acid and sulphate ester (sialosulphoglycoproteins) or with neutral ends (neutral glycoproteins). These ends determine the extent of negative charges on each mucin molecule [17].

Intestinal secretory mucins are synthesized and secreted by goblet cells, a specialized wine-goblet-shaped epithelial cell lineage dispersed along the intestinal lining. The dimerization and/or polymerization of mucin molecules and the electrochemical properties of mucopolysaccharides are believed to determine the chemical and biophysical characteristics of mucus along the GIT [18].

Mucins have a key role in avoiding potential damage from microbes. The mechanism by which mucus controls microflora colonization is referred to as part of innate epithelial cells [19]. The role of mucin on microbe colonization is manifested in at least two distinct ways. First, some microbes are mucolytic, including Bacteroidetes, and use mucin glycoproteins and carbohydrates as an energy source and provide physical support for intestinal colonization. Moreover, these bacteria provide substrates for other bacteria in the outer mucus layer by degrading the mucins [20, 21]. Second, mucins are generally "toxic" to the proliferation of certain microbes. Mucus gel inhibits proliferation by entrapping microbes that are starved or killed by antimicrobial peptides, and/or expulsed by the luminal flow. Mucus also provides a physicochemical barrier to prevent microbes from direct contact with epithelial cells.

Moreover, the mucus gel provides a matrix for antimicrobial molecules, which are mainly produced by Paneth cells. Direct interactions with mucins can facilitate the diffusion of these antimicrobial molecules [22]. Taken together, mucins have been proposed to play an important role in shaping microbial communities at the intestinal mucosa. Recent studies suggest the correlation between changes in mucin glycosylation profile and deviations of overall microbial community ecology as well as altered abundances of specific microbes [23, 24].

3.2 Trefoil factors

Co-expressed with mucin-secreting cells and in close relation with mucus, trefoil factors (TFF) demonstrate an interesting group of mucus molecules. Trefoil factors were initially discovered in the pig pancreas [25] and further characterization of this family has strikingly observed their abundant expression in the GIT and their efficacy as therapeutics especially for preventing and treating various GIT conditions [26, 27]. They are named as trefoil by their "three-leaf" structure and are a family of small (7-12 kDa in mammals) protease resistant peptides whose common unit is the trefoil motif [25].

It is now clear that TFF participate in the healing of mucosal injury in disease conditions by promoting cell migration over damaged areas (rather than promoting cell division), and inhibiting cell death, and are also believed to be involved in physiological repair of epithelia from daily apical sloughing against frequent luminal insults [25, 28, 29].

TFF have recently been found to participate in immune responses. It was showed that TFF2 deficiency or administration of recombinant TFF2 altered the expression of immune associated genes including defensin genes in Paneth cells [30]. The presence of TFF in immune organs, including spleen, thymus, lymph nodes and bone marrow [31], may suggest possible regulatory role(s) played there. TFF can be a potent mitogen by regulating chemotaxis, stimulating the migration of immune cells. The molecular basis of such may be supported by the recent in vitro evidence that recombinant TFF2 activates CXCR4 chemokine receptors and attenuates CXCR4 mediated chemotaxis [32]. This finding also highlights a molecular linkage between TFF and the immune system.

TFF are thought to cooperatively interact with mucins in the lumen to enhance the protective barrier properties of the adherent mucus layer against bacterial and toxic insults [25, 28]. Thim et al. [33] observed significant increase in the viscosity and elasticity of gastric mucin solutions because of TFF2 addition [33]. Increased viscosity could help prevent antigens from approaching the epithelium surface, especially in healing epithelia, which eventually benefits epithelium restitution and alleviates immune system burden. In this scenario, TFF are predicted to be involved in mucus polymerization.

3.3 Goblet cells

Goblet cells together with absorptive enterocytes, Paneth cells (secreting antimicrobial peptides etc.) and enteroendocrine cells, represent the four principal cell types that are continuously renewed in the epithelium of the small intestine. During intestinal epithelial cell regeneration, pluripotent stem cells that reside at the bottom of the crypt divide to generate multiple cell lineages which migrate from the proliferative crypts to the villus tip [34]. While migrating along the crypt-to-villus axis, goblet cells are terminally differentiated from secretory cell lineage derived from a common Math1-expresing progenitor cell [35]. Goblet cell differentiation is controlled by winged helix transcription factors Foxa1/a2 which can also transactivate Muc2 promoters [36].

It is generally believed that goblet cells producing neutral mucins contain little sialic acid and represent an immature state; while goblet cells containing acidic mucins are more likely resistant to infections because they are normally "upregulated" in response to bacterial infection. In addition to mucins, several other molecules are co-expressed within the intestine such as ingobsin (localized in human and rat goblet cells) with endoproteolytic activity in the presence of both epidermal growth factor and cobalamin-binding protein haptocorrin [37]. TFFs are (specifically TFF3) along with mucins biomarkers of goblet cells.

3.4 M cells

M cells or Microfold cells (because of uneven microvilli) are classified as epithelial cells with large fenestrations in their membranes. These features enhancing the uptake of antigens from the gut lumen [38]. They have a capability for capturing luminal antigens and transporting them across the epithelium ("transcytosis"). They are placed in the gut epithelium called follicle associated epithelium overlying the domes of Peyer's patches and other lymphoid organs. M cells are not professional antigen-presenting cells because they do not have the ability to process and present antigens to the major histocompatibility complex (MHC) molecules. Instead, they serve as antigen delivery cells, that is, as a functional equivalent to lymphoid nodes because they provide antigens to professional antigen-presenting cells, such as dendritic cells (DCs), macrophages as well as B lymphocytes. Indeed, many pathogens take advantage of their transport efficacy to invade the body [39–41]. M cells subsequently transfer these antigens to underlying DCs enabling the transfer of captured molecules through transcytosis mechanism (which remain to be elucidated) as well as intracellular material through microvesicles to underlying DCs [42]. In conclusion, M cells provide specialized full-service immune surveillance capabilities.

3.5 Paneth cells

Paneth cells are physiologically found at the distal small intestinal crypts of Lieberkühn and contain abundant secretory granules. Their unique histo-morphological features implicate special functions in cellular homeostasis as well as in the establishment and configuration of the mucosal barrier as a physical and highly organized immune interface [43]. Previous studies suggesting the existence of Paneth cells in the chicken remained controversial. However, recent research has supported Paneth cells existence in the small intestine of the chicken by electron microscopy confirming the presence of granulated secretory cells at the base of the crypts in the chicken small intestine. The researchers also confirmed by Western blot the expression of lysozyme protein, which is specifically secreted by the Paneth cells in the small intestine [44]. Paneth cells have the morphological characteristics of a professional secretory cells, including an extensive ER, a Golgi apparatus and an internal secretory granule. The first assumption that Paneth cells had a host-defense function emerged when lysozyme was identified as a product of these cells [45]. After that, it was discovered that Paneth cells secrete antimicrobial peptides (AMP) or host defense peptides (HDPs) which are important host-defense substances in the communication between host and microbiome. One of the most well characterized are β-defensins [46]. In addition to defensins, Paneth cells is able to secrete other AMPs including secretory phospholipase A2, Reg III, angiogenin 4 and cathelicidins [47–49].

3.6 Host defense peptides

HDPs are generally positively charged small peptides with amphipathic properties [50]. These peptides present in the GIT display an important, but often overlooked role in the first line of defense. With the first avian HDPs identified in 1990s [51], the information about avian HDPs has increased considerably in the subsequent decades. Currently, avian β-defensins and cathelicidins are the two major classes identified and extensively studied in chickens [52, 53].

HDPs were initially called antimicrobial peptides (AMPs), because they are characterized by the direct antimicrobial activities against a broad spectrum of numerous pathogens, including gram negative and positive bacteria, fungi, and even certain viruses [54–56]. Generally, the cytoplasmic membrane of pathogenic organisms is a frequent target for HDPs. The amphipathicity and cationic charge of HDPs allow the initial contact with membrane electrostatically, as most bacterial surfaces are hydrophobic and anionic. The peptides then insert into phospholipid bilayers and induce pore formation in membranes by toroidal pore formation, carpet formation and barrel-stave formation, resulting the cytoplasmic leakage and death of pathogens [54, 57–59]. Besides pore formation in membranes, some HDPs can directly penetrate into cells and interfere with intracellular molecules, interrupting cell wall formation, DNA and RNA synthesis, protein translation and post-translational modification [57, 60].

To be specific, chicken AvBD1, −2, and − 7 exhibit high efficiency against a large variety of both gram-negative (*E. coli*, S. enteritidis, S. typhimurium, C. jejuni, and *K. pneumoniae*) and gram-positive (*S. aureus*, B cereus, *L. monocytogenes*, S. haemolyticus, and S. saprophytus) bacteria [51, 61–64]. AvBD1 and − 7 also efficiently kill P. aeroginosa and E. cloaca, while AvDB2 showed reduced efficacy [61, 64]. AvBD4, −5, and − 11 protect host from invasion of S. enteritidis and S. typhimurium, however their antimicrobial activities on other bacteria species remain to be determined [63, 65, 66]. Although AvDB8, −9 and − 13 are active against *E. coli*, respectively, they exhibit a minimal activity against several other bacteria [66–69]. Based on studies of different AvBD isoforms, it seems that both structure and catholicity are important for antimicrobial activity but disparity in the preference of gram-negative or positive bacteria.

All four chicken CATHs show antimicrobial capacities in the same order of magnitude against a wide range of gram-negative and positive bacteria, and fungi [70–73]. Similar to AvBDs, the structure and cationic charge are equally important for their antimicrobial activities. The presence of an alpha-helical region in N-terminal and hinge region around the center of the peptide are important for antimicrobial. Removal of N-terminal alpha-helix in CATH2 truncation or disrupted helix formation in a-helical synthetic peptide leads to the loss of antimicrobial activity [72, 74, 75]. Although deletion of C-terminal alpha-helix in CATH2 reduces the activity against pathogens, the remaining truncation is still capable to kill bacteria [75]. The truncation of CATH2 with N-terminal alpha-helix alone shows increased antibacterial activity [76]. The hinge region plays a key role in the insertion of CATH into the bacterial membrane and pore formation [74, 77]. Disruption of the hinge region by point mutation or removal in the center of the CATHs largely decreases the antimicrobial activity [72, 74, 78]. The cationicity of CATH and AvBDs is important for the initial contact with the surface of bacteria. The higher cationic charge in CATH2 and the synthetic analogs results in the better antimicrobial outcomes [72, 75].

In addition to direct antimicrobial activity, the HDPs exhibit the immunomodulatory function, involving inflammation and chemotaxis. Chicken AvBD13 was reported as a direct TLR4 ligand [79], increases production of IFN-γ and IL-12 in mouse monocytes through activation of TLR4-NFκB axis. Combined with the evidence that AvBD13 increases serum IgG and IgM levels in chicken and induces lymphocytes proliferation in spleen after the administration of the infectious bursal disease vaccine (IBDV) [80], activation of TLR signaling by AvBD13 indicates an immune enhancement rather than a merely pro-inflammatory effect. Moreover, chicken AvBD1 fusion protein expressed by IBDV enhances CD4+, CD8+, and CD3+ T-cell proliferation, increases antibody titers, improves survival rate in in vivo experiment [81]. Additionally, HDPs have been shown chemotactic effect. Investigations about immunomodulation by avian AvBDs and CATHs are mainly limited to NF-κB activation, cytokine production, and direct immune activation. The similar findings in human and mouse studies suggest the conserved function of HDPs among species, providing the guideline for the application and future research in poultry area.

4. Adaptative immunity of the GIT

Unlike the innate immune system which attacks only general threats, adaptative mucosal immune system is triggered by exposure of potentially dangerous pathogens. However, sometimes if overlaps some of their functions. The three most key roles of that system are: the induction of an efficient and appropriate immune response to pathogenic invaders, the tolerance of the commensal microorganisms of the intestine as well as the induction of the tolerance of nutrients and other environmental immunogens. Responses of the systemic immune system can originate from or be modified by the mucosa; this is exemplified by the attenuation of systemic immune responses to a protein that has first been fed orally to the animal (oral tolerance). Thus, the mucosal immune system must maintain the delicate balance between responsiveness to pathogens and tolerance to a vast array of other harmless antigens encountered at mucosal sites. This balance is achieved through the interplay of innate and adaptive (B- and T-lymphocyte) mechanisms [82].

The adaptative immune system in the GIT has features that are distinct from adaptative immune systems in other organs. The major form of adaptative immunity in the gut is humoral immunity directed at microbes in the lumen. This function is mediated mostly by dimeric IgA antibodies that are secreted into the lumen of the gut. Cellular adaptative immunity is carried out by an intraepithelial lymphocytes (IEL) in healthy adult bird includes major subsets of NK and T cells bearing the γδ or αβ form of the T cell receptor (TCR). In contrast to other tissues, B cells are almost entirely absent from the IEL and the T cells predominantly express the CD8 co-receptor with smaller populations of TCRαβ+ CD4+ and CD4 + CD8+ cells [83, 84].

Moreover, within the CD8+ IEL population the majority express CD8αα homodimers rather than the CD8αβ heterodimer commonly expressed on classical CD8+ T cells found at systemic sites [83–85]. The proportions of IEL belonging to each subpopulation differ according to age, genetics and environment (including infection). Numerically, B and T cells are the most common lymphocytes (~90%), the remainder being of the NK cell phenotype (CD3-Bu-1-). In contrast to the IEL population, the T cell population of the lamina propria contains a smaller proportion of γδ-T cells (~10%); the much larger αβ-T cell population is dominated by CD4+ T cells, with a less prominent CD8+ cell population [86].

4.1 Secretory IgA (sIgA) and its transporter, polymeric Ig receptor (pIgR)

The existence of sIgA in the bird has been established for quite some time, but studies are relatively limited compared with mammals. In humans, it is estimated that approximately 70% of the body's IgA-producing plasma cells (differentiated from activated B cells) reside in the lamina propria of intestinal mucosa [87–89]. Although sIgA belongs to adaptive immunity by definition, it plays an important role in the first lines of mucosal defense [87, 90]. There are three modes of defense modulated by sIgA on gut mucosal surfaces: (1) sIgA has been shown to interfere with the early steps of the infection process through directly blocking pathogens and toxins from attaching to the intestinal epithelium [91]; (2) sIgA exerts the protective immunity through immune exclusion, which is the prevention of pathogens and toxins from approaching to epithelium through the stepwise procedures involving antibody-mediated agglutination, entrapment in mucus, and clearance through intestine peristalsis [92, 93]; (3) sIgA exhibits the ability to neutralize intracellular pathogens, viruses, and toxins within intestinal epithelial cells, which requires binding of specific IgA and occupation of antigens by pIgR transportation vesicles, followed by the passage of antigens into the lumen. Notably, the intracellular neutralization of LPS by IgA indicates the potential role in anti-inflammation and deactivation of the proinflammatory pathways in epithelial cells [90, 94, 95].

T-cells generally produce high-affinity IgA antibodies. IgA has the specificity against previous exposure of the GIT by pathogens and more invasive commensal species [89, 96]. Conversely, low-affinity IgA antibodies can also be produced from T-cell-independent (TI) pathways. These low-affinity IgA antibodies act through coating commensal bacteria thereby augmenting the competitive inhibition of pathogens [88, 89, 96]. The production of both high and low-affinity production of IgA provides protective roles during an overt infection with a pathogen as well as during unchallenged/non-pathogenic bacterial exposure.

Presence of microflora in the the GIT may also regulate production of IgA. Studies with germ-free mice [99] and pigs [100, 101] have demonstrated that intestinal IgA and IgA-positive cells in the lamina propria are dramatically reduced versus conventionally reared animals. Further studies have shown that specific microflora (e.g. segmented filamentous bacteria and clostridia) when given to germ-free mice will stimulate the development of IgA-producing cells, while other microflora will have no effect or inhibit this development [97, 98]. Thus, other researchers have reported similar IgA production responses with poultry diets were supplemented with probiotics [99, 100]. Notably, IgA development in the hindgut of the bird early in life coincides with the rapidity of bacterial colonization [101].

Prior to development of IgA by the GIT, the chick is reliant upon maternal antibodies and physical defenses (such as mucins and intestinal turnover). In birds, a small amount of IgA (~ 0.3 mg) is transferred via the embryo imbibing amniotic fluid prior to internal pipping [102, 103]. The endogenous IgA expression starts to increase after the second week post-hatch [101]. Bar-Shira et al. [103] suggested that the resistance of rapid depletion of maternal IgA may be due to unique uptake by goblet cells, which serves as a reservoir to slowly release maternal IgA.

Circulating IgA is predominantly in a monomeric form, whereas in intestinal secretions it is found in a dimeric configuration both in mammals and birds [96, 104]. IgA secreted by plasma cells accumulates in the lamina propria. To exert its protective effect, pIgR is constitutively expressed by epithelial cells to transport IgA through the epithelia from the lamina propria to intestinal lumen. During the transcytosis, IgA is bound by pIgR on the basolateral surface and transported to the apical surface. At this surface, cleavage of the extracellular portion of pIgR results in release of secretory component (SC) as part of the dimeric IgA, otherwise known as the sIgA complex [105]. In this complex, sIgA is thought to be protected against degradation by proteases and pH fluctuations in the gut [90]. Excess production of pIgR which is not utilized as an IgA chaperone is also secreted as "free SC", which may have additional bacterial scavenger properties [106]. Once secreted, the N-glycans of SC can then bind to itself, and/or sIgA in the mucin layer thereby bridging these luminal defenses [105].

As one molecule of pIgR is required to bind and transport one dimeric IgA for secretion of sIgA into the intestinal lumen, pIgR's expression regulates sIgA capacity into the GIT [105, 107]. Expression regulation in mammals can be induced by numerous cytokines, including: interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-1β (IL-1β), and IL-4. These cytokines act through mediating a transcriptional response through activation of several transcription factor-binding sites in regulatory regions [105, 107–109]. In the chick, increases in IFN-γ, IL-1β and IL-4 expression in the second week post-hatch [110, 111] may influence subsequent increases in expression of the chicken pIgR gene [111]. Additional bacterial binding to Toll-like receptors have also been shown to increase pIgR expression in epithelial cells [105, 107, 112].

5. Nutrition and secretory immune response

The GIT is an extremely expensive tissue in terms of energy and nutrient needs to maintain and facilitate the full range of barrier and energy/nutrient assimilation functions it displays. Cant et al. [113] estimated that the GIT consumes approximately 20% of dietary energy with a turn-over rate of 50 to 75% per day. However, the GIT is a dynamic organ system whose maintenance needs dramatically changes based on responsive demands. Applegate [114] elucidated some of these adaptive responses, including: changes to peristaltic rate, changes to enterocyte turnover, tight junction regulation, mucin production (quantity and composition), changes to differentiation direction of undifferentiated cells and changes to secretory defenses.

While we often think of presence of microbiota as an additional barrier cost, there is some symbiotic relationships that they convey to the host. For example, the presence of the ceca contributes approximately 3% to dry matter digestibility to the bird [115] in part through 8% of energy derived from microbial fermentation resulting in short-chain fatty acids [116, 117].

Due to limitations of space in this review, we were unable to address all nutrient impacts on the secretory immune defenses of the bird. Notably, recent reviews have published on roles of amino acids on physiological, immunological, and microbiological responses as well as quantification of changes to endogenous amino acid production in the bird [118, 119]; as well as implications of protein indigestibility in the GIT and implications of microbial fermentation of protein in the hindgut of the animal [120]. Additional impact of microminerals (e.g. zinc, copper, and manganese) and plant bioactive compounds on intestinal functionality have been elucidated [121, 122]. Similarly, recent research has revealed modes of action of specific classes of feed additives that directly or indirectly influence the secretory immune responses of the GIT. For example, probiotic and phytogenic additives have had numerous reviews on these actions [123–125]. Further elucidation of contribution of specific fibrous and fatty acids to the intestinal secretory defenses are further elucidated.

5.1 Dietary fiber and intestinal health

Carbohydrates that are not hydrolyzed by endogenous enzymes in the upper GIT can be fermented by bacteria in the large intestine and ceca are designated as dietary fiber [126]. Dietary fiber (DF) resides in the indigestible portion of plant derived foods that include cell walls, non-starch polysaccharides (NSP), oligosaccharides and lignin [126, 127].

Polysaccharides of NSP include cellulose, pectins, β-glucans, pentosans, heteroxylans and xyloglucan [128]. There are two different types of NSP, soluble and insoluble. Such classification is based on their solubility in water. The ability of soluble NSP to mix with water, producing an increase in the viscosity of the digesta and decreasing the binding of digestive enzymes, negatively affects the digestibility of nutrients [129]. As a result of suboptimal digestion, there is an increase in GIT surface area and secretion of digestive enzymes, creating an increased endogenous energy cost of digestion and affecting bird productivity [130]. NSP from cereal-based diets are associated with low apparently metabolized energy, increased feed conversion rates and increased incidence of wet droppings.

Some previous studies have considered the effects of different cereal NSP based diets on the intestinal microbial immunity. Different types of cereal can modify specific members of the microbiota in the cecum of chickens in two different ways; by altering the viscosity and pH and/or by supplying nutrients to produce the selective growth of specific bacteria [131]. The increase in digesta viscosity with the subsequent reduction in feed passage rate leaves more undigestible feed in the intestine, which represents an ideal substrate for bacterial growth [131]. Chickens fed with a barley-based diet had a higher number of *Clostridium perfringens* in the ileum and ceca. Likewise, it has been reported that the use of wheat in poultry diets may favor the proliferation of pathogenic bacteria like *Escherichia coli*, Salmonella and Campylobacter [132].

In contrast, insoluble NSP is metabolized into short chain fatty acids (SCFA) including acetate, propionate, butyrate, valerate and isovalerate [116]. Those fermentable metabolites serve as sources of carbon and energy for the commensal microbiota in the lower intestine, specifically, for the bacterial population in the ceca of chickens [116] which provide up to 10% of the energy to the bird. In addition, cecal reverse peristalsis produces translocation of the cecal microbiota affecting energy metabolism and performance [133]. The fermentation metabolites produced by the intestinal bacteria depends on the availability of the substrate, fermentation mechanisms and bacteria specie involved in the process [117].

Dietary fiber has a direct, positive effect on the immune response in numerous species by increasing the abundance of some immune cells, specifically T cells, in the gut-associated lymphoid tissue [134], changing the cytokine secretion profile [135, 136] increasing mucosal immunoglobulins and by acting as a prebiotic substrate for beneficial bacteria [137]. For feed ingredients to be considered prebiotics, they have to meet the following criteria: resistance to an acidic environment (indigestible), fermentation by intestinal microbiota and selective stimulation of beneficial bacterial populations [138]. Based on this concept, dietary fiber is classified as a prebiotic.

A number of studies have found that fiber-rich prebiotics can enhance immune function including direct production of SCFA [139, 140], augmentation of gut burrier function [141], influence on immune mediated inflammatory responses and restoration of the physiological function of bacterial populations.

In human nutrition, multiple benefits have been attributed to dietary fiber, including maintaining normal bowel structure and function, increasing water retention, blood flow, fluid, and electrolyte uptake in colonic intestinal mucosa [128, 142]. Moreover, fiber intake can reduce the risk of metabolic diseases such as obesity, coronary artery disease, diabetes, constipation, inflammatory bowel disease, colitis and colon cancer [128]. In diets rich in protein, the inclusion of dietary fiber such as arabinoxylan-oligosaccharides (AXOS) can potentially decrease the generation of toxic metabolites originated from proteolytic activity and increase the amount of health-promoting bacterial populations [143].

The addition of dietary fiber has also been widely adopted in swine nutrition in order to maximize the nutrient supply and intestinal health [144, 145]. Dietary fiber can change the physiological features of the digesta, most notably modifying the transit time, and the composition of digesta in terms of solubility, fermentability and water retention. Such changes have a direct impact on intestinal functions, bacteria population and fermentation. The inclusion of high to moderate levels of dietary fiber in pigs, remodel the gut microbiota since certain healthy bacteria species such as Lactobacilli and Bifidobacterium tend to increase. The proliferation of lactic acid producing bacteria decrease the pH of the intestinal lumen, resulting in decreased abundance of other pH sensitive enteropathogenic bacteria like *Escherichia coli*, Salmonella, Shigella and Clostridia [144, 145]. Other effects of dietary fiber have been demonstrated. Changes in the gut morphology, most remarkably inducing increases in crypt depth and altering cell division in growing pigs. This effect has been attributed to the trophic nature of SCFA, specifically butyrate [145]. In contrast, fiber is a feed ingredient poorly utilized in poultry nutrition due to antinutritional effects observed from soluble fiber sources that are mainly associated with increased viscosity of digesta and subsequent impair of nutrient absorption and performance parameters [129]. The effects of fiber are variable and depends on the fiber source, particle size, level of inclusion and chemical composition [146]. A number of studies have found that low levels of insoluble fiber can provide benefits from the point of view of gut health by improving nutrient digestibility [147], gizzard functionality, and resulting in modulation of digesta passage and higher nutrient retention [148, 149]. In the literature, a wide range of other effects of dietary fiber have been demonstrated in laying breeders and broilers chickens. In commercial layers supplemented with high fiber ingredients in the diets, environmental improvements have been demonstrated by reducing ammonia concentrations in manure [150], feather pecking [151], cannibalistic behavior and associated mortality [152].

A number of oligosaccharides including lactulose, inulin, galacto-oligosaccharide and mannan oligosaccharides have been proposed to use as prebiotics in chickens. Those non-digestible carbohydrates are metabolized by fermenting bacteria to produce SCFAs. SCFA are nutritional substrates required for an adequate function of the immune system [139]. When xylo-oligosaccharides were supplemented into a broiler chicken diet, the abundance of butyrate-producing bacteria in the colon and ceca, such as Bifidobacterium and Lactobacillus, significantly increased [153].

Similarly, Zhao et al. reported an increase in Lactobacillus counts in excreta when birds were fed with 0.15% inclusion of lactulose [154].

Production of butyrate is considered advantageous to maintain gut health. Butyrate is an important energy source for the enterocytes [140] and is characterized for having immunomodulatory properties. Butyrate can have an anti-inflammatory effect by modulating key inflammatory mediators including the reduction of IFN-γ and NF-kB and the increase in the number of T reg cells and the expression of IL-10 which suppresses the activity of the immune system [155]. Likewise, inulin supplementation in broiler chickens (0.25-0.5%) induces an anti-inflammatory response by decreasing the gene expression of proinflammatory cytokines such as NF-kB, LITAF, IL-6, iNOS and enhances the protective barrier function represented by increased expression of epithelial tightness components including MUC2 and claudin-1 [156].

Other major effects have been shown with the supplementation of oligosaccharides, such as the improvement of growth performance [153], the influence on the intestinal morphology reflected in an increase in crypt depth, villus height and villus area [157] and the reduction of pathogenic bacterial colonization. The increase in pathogen resistance due to prebiotic supplementation is associated with the simultaneous elevation of lactic acid producing bacteria and the decrease in the pH of the intestinal lumen, creating an unfavorable environment for pathogenic bacteria and thereby decreasing the colonization. In fact, a meta-analysis study showed a reduction of 0.61 log10 cfu/g cecal Salmonella spp. in birds treated with lactose and its associated prebiotic products (lactulose, lacto-sucrose, whey and dried milk) [158].

5.2 Fatty acids and immune response

Short-chain and medium chain fatty acids play an important role on maintaining intestinal gut health and controlling enteric pathogens [159]. Endogenous metabolic pathways, including beta oxidation of fats, leads to the production of short chain fatty acids (SCFA) such as acetate, propionate and butyrate [160]. Long chain fatty acids can be converted into acetate via acetyl-coA or in propionate via propionyl-CoA [160]. SCFA can modulate multiple cellular metabolic activities by the interaction of nuclear cellular (G-protein couple receptors: GRPs), enzymatic receptors (histone deacetylases: HDACs), serving as a substrate for energy for enterocyte and Krebs's cycle and inducing apoptosis of cells [156]. Through these mechanisms, SCFA modulates gene transcription of cells involved in metabolic pathways, inflammation and immune response. In intestinal cells, butyrate and propionate has the ability to inhibit the HDAC activity which decrease the activation of NFkB transcription factor and subsequently modulating the expression of inflammatory genes [161]. The anti-inflammatory effect of butyrate is produced by preventing the secretion of pro-inflammatory cytokines by macrophages through the NFkb pathway [156].

Regarding the adaptive immune response, butyrate plays an important role in modifying various lymphocyte function including the inhibition of T-cell proliferation, and reduction of the secretion of pro-inflammatory cytokines such as IL-2, IFN-γ and promoting the production of the main anti-inflammatory cytokine, IL-10 [156, 161].

Due to its anti-inflammatory properties, SCFA has been used as a therapeutic alternative for intestinal diseases [162]. Direct delivery of SCFA by encapsulation allows the supplementation without the need for fermentation, increasing the release in the distal gastrointestinal section [163]. Multiple studies have shown that SCFAs are beneficial as a drinking water supplement and feed additive for the control of Salmonella, Campylobacter and Clostridium [164, 165].

In young chickens, *Salmonella enterica spp. enteritidis* cecal colonization significantly decreased when butyric acid was added to the feed [166, 167]. The addition of SCFA in the drinking water has also been used as an efficient strategy for decreasing the recovery of *Salmonella enterica spp. typhimurium* in the crop and in pre-chilled carcasses at the processing plant [168]. The reduction in colonization of Salmonella by SCFA is related to the regulation of invasion genes (hilA, invF and sipC) located on the pathogenicity island (Sp1-1) [169]. In addition to the antimicrobial activity, SCFA can contribute to disease resistance by enhancing the expression of host defense peptides including Avian β-defensin 9 (*AvBD9*), *cathelicidin B1*, *AvBD3*, *AvBD4*, *AvBD8*, *AvBD10* and *AvBD14* which consequently reduce bacterial growth [170]. However, the ability of SCFA to control Salmonella is highly correlated with acid type and concentration. For example, the feed supplementation of butyric acid in the coated form is more effective in decreasing *Salmonella enterica spp. enteritidis* counts than when using the powder form [166]. Previous studies have also investigated the formic-propionic acid combination at 0.5 and 0.68% respectively, with a significant reduction of *Salmonella enterica spp. kedougou* [171]. Furthermore, the use of a combination of propionic and formic acid decreased the recovery of *Salmonella enterica spp. typhimurium* in the ceca by 3.61 log at 21 days [172]. Similarly, the combination of 1.5% of formic acid and 0.1% of sorbic acid were protective against Campylobacter jejuni colonization during infection trials in broiler chickens by reducing C. jejuni counts in the crop [173].

Among different classes of fatty acids, medium chain fatty acids (MCFA) have reported to be more inhibitory against Salmonella than short chain fatty acids [163]. MCFA are fatty acids composed by 6 to 12 carbons and include caproic, caprylic, capric and lauric fatty acids [174]. The greater antibacterial effect of MCFA is corelated with metabolic differences. Because of its smaller molecular size, MCFA can be absorbed more efficiently and therefore can be utilized more efficiently in the intestinal tract [175]. Indeed, the in-vitro antimicrobial activity of MCFA against Salmonella is observed at very low concentrations (between 10 nM- 50 nM) [176, 177]. Furthermore, in-vivo studies have shown reduction in Salmonella cecal counts with supplementation of caprylic acid [178, 179]. The supplementation with either 0.7 or 1% of caprylic acid significantly reduced the *Salmonella enterica spp. enteritidis* counts in cecal samples of birds fed caprylic acid 7 to 10 days post-challenge in 18 day-old chickens [179]. Another study, showed a reduction in cecal Salmonella *Salmonella enterica spp. enteritidis* counts in ceca, spleen and liver [178] in 3 and 6-week-old chickens. Similarly, the supplementation of caproic acid in broilers decrease the colonization of Salmonella through hilA gen suppression [177].

MCFA acid have also been used for controlling Campylobacter jejuni. Although, studies have shown inconsistent results, caprylic acid at 0.7 and 1.4% has shown to be effective in reducing C. jejuni counts by 3 to 5 log in infected chickens [180].

In conclusion, the application of fatty acids to reduce inflammation and intestinal pathogens is an alternative strategy for poultry nutritionists. Multiple studies support the important role of fatty acids as a modulation of intestinal health. Long chain fatty acids can modulate innate and adaptive immune responses and reduce inflammation produced by systemic diseases. On the other hand, SCFA and MCFA modulate the immune cell function to facilitate the elimination of pathogenic bacteria. Understanding the role of fatty acids in health and disease will increase the effectiveness of these compounds in a wide range of intestinal, metabolic and inflammatory diseases.

6. Conclusions

In summary, secretory defense host response and their players including host defense peptides, sIgA and pIgR among others, constitute the first line of intestinal immune defense and bridge innate and adaptive immune responses at mucosal surfaces. Understanding the complex function and regulation of these immune components may offer new insights into the nutritional prevention and treatment of infectious and inflammatory diseases that originate at mucosal surfaces. Some studies have been addressed the role of key nutrients modulating this secretory defense system and aiding to the host to counteract the noxious effect of harmful microorganism. Based on that, nutrition would be considered as an important strategy in the reduction of antibiotic growth promotants. However, more studies are needed to understand the effects of nutrients on gut immune response against pathogens.

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Chapter 9

Probiotics as a Promising Additive in Broiler Feed: Advances and Limitations

Celina Eugenio Bahule and Tamiris Natalice Santos Silva

Abstract

Feed additives have a strong influence on the production cost of broilers as growth-promoter's to cover variations in profits due to fluctuation in feed costs. Antibiotics as additives were fundamental and indispensable, however, studies have shown their connection with the emergence of resistant strains of pathogens in animals and humans, therefore in recent years they have been less encouraged. Research is in progress concerning additives that can replace antibiotics as growth promotors and also as prophylactics. It was demonstrated that probiotics, which are living microorganisms and without residual effect's have a potential to be used as microbials. However, they are not always guaranteed as growth promoters, as there are mechanisms of action regarding their interaction with the host that cannot yet be properly understood. The main advances in the use of probiotics in broilers in recent years, as well as the gaps, challenges, and future perspectives were carefully discussed and analyzed in this study. It was considered as a future premise, the possibility of reviewing the traditional methodologies used to test the hypotheses related to the effect of probiotics in broilers, which may also be extended to other animal species.

Keywords: feed additives, intestinal health, use of nutrients, antibiotics, performance

1. Introduction

Broiler chicken is an affordable and complete source of protein for human consumption. In addition, it is a source of income for families, small and large companies around the world [1, 2]. In broiler breeding, intestinal health is crucial, due to its influence on health and use of nutrients [3]. Furthermore, its influence directly in obtention of the ideal performance parameters. Special attention must be paid to the gastrointestinal tract, as it is where most disease-causing pathogens enter, settle and multiply [4]. In the same way preventing or treating diseases of the tract results in more efficiency for other bird body systems [5].

After hatching, the intestinal tract of birds is sterile, which becomes populated with microorganisms when searching for food, constituting their intestinal flora [3]. Intensive breeding coupled with confinement applied in modern production of broilers limits the movement of birds putting them at risk of health vulnerability and dependence on the provided diet [6].

Various additives with different functions have been used to formulate complete diets for broilers. Some additives act as nutrition increments' and others guarantee protection against disease. Antibiotics were among the most used additives to control the birds health [7].

Antibiotics were widely used as growth promoters, whose application was made in sub-therapeutic doses throughout the breeding of broilers. However, strong evidence indicated that antibiotics contributed to the development of resistance in pathogenic strains [8, 9].

The emergence of resistant strains in poultry farms have been rousing a major concern. Reports have proven not only the damage on the animal's health but also to human due to the trophic interactions [9, 10]. Consequently, there are major concern to withdraw antibiotics from broiler chickens. The search of alternative sources of additives that would be potential substitutes for antibiotics in broiler chickens become a crucial issue for many researchers [11]. Nonetheless, the use of antibiotics presents economical concerns, because beside to aforementioned advantages they acted in the control of diseases and in reducing the burden of carcass contaminants [12].

Probiotics became a potential antibiotic substitute additive in broilers, due to its influence in performance of broilers and security compared to antibiotics [13]. Probiotics are groups of non-pathogenic microorganisms, normally inhabitants of the intestinal flora, which, when administered have the benefit of balancing the intestinal microbiota by inhibiting the excessive multiplication of pathogenic microorganisms [3]. In addition, the presence of these in the tract activates the host's immune system, stimulating them to remain alert against any invasion [14].

Probiotics had already been applied to humans and other animal species. Certain species of microorganisms among which bacteria, fungi, and yeasts are carefully selected to be used as probiotics [15].

In broiler, however, even today, it is still not possible to accurately predict the effect of probiotics, due to several intrinsic and extrinsic factors that interfere with their use and results tend to be different between studies. The purpose of this study is to critically analyze the main findings regarding to probiotics applications in broilers.

2. Probiotics in broilers, concept, types and effect

Probiotics in animals as well as in humans are being extensively studied, due to its effect on the restoration of the intestinal microbiota, reversal of dysbiosis (gastrointestinal imbalance), and safety in its use [16, 17]. The use of antibiotics is banned from the European Union (EU) and other parts of the world, catalyzed the research of probiotics for broilers. In addition, the antibiotics ban, not only affected countries importing meat but also pressured the meat exporting countries to meet the regulations of receipt countries [10, 18].

The use of antibiotics in animals is prohibited in the EU and the USA, is the practice responsible for creating antibiotic-resistant bacteria, known as superbugs. These superbugs having animals as reservoirs can spread among other animals, management personnel, food and the environment [12].

Some portfolios have been developed possible alternatives to antibiotics [11], these have included probiotics in the most advanced approaches to serve as preventive, and alternative therapies with potential uses in animal health. Derived from Greek, the word probiotic means "pro-life" and in the search to cover the correct mechanism of probiotics, this term has undergone several changes over the years. In animal production, it can be summed up as a "feed supplement for live microorganisms that beneficially affects animal flora by improving the microbial balance in the intestine" and should be viable and stable in the different breeding conditions [19].

The most used microorganisms as feed supplements are mainly bacteria, mostly from the group of Gram-positive *Bifidobacterium* and lactic acid bacteria (LAB). The genus *Enterococcus, Lactobacillus, Pediococcus, Streptococcus, Lactococcus*, and *Leuconostocos,* and also *Bacillus* are the most used. In addition to bacteria, fungi and yeast strains, mainly from the species of *Saccharomyces cerevisiae* and *kluyveromyces* are also used as probiotics [8, 19, 20]. A microorganism to be considered as a probiotic must meet some requirements: (1) to resist and quickly occupy the intestinal tract; (2) be part of the intestinal habitat; (3) survive the action of digestive enzymes; show antagonistic action against pathogenic microorganisms;(4) be non-toxic and non-pathogenic; (5) be stable and viable in commercial preparation and stimulate the immune system [21].

Several probiotic strains are included in poultry diets to promote animal growth and health, especially when conditions are challenging for health. Several studies have reported the beneficial effects of probiotics on various aspects of bird health. Furthermore, parameters related to supplementation with probiotics in diets increased body weight gain, feed intake and improved feed conversion rate in birds, were also reported (**Table 1**).

Until now, many mechanisms about the action were proposed. However, the main mechanisms of action of the probiotics are competition for binding sites, where the probiotics adhere to the intestinal epithelium wall, hindering competition and the joining of pathogenic microorganisms, this higher concentration of the beneficial microbiota also causes it to have advantage in competition for nutrients [12]. Supplementation with *Bacillus subtilis* improves the performance and immunity of broilers reared in warm conditions, and birds fed probiotic in the diet were able to deal more effectively with heat stress through immunity modulated by the

Parameters evaluated	Probiotic strains used	Source/Author
Promotion of feeding efficiency and improvement of subclinical necrotic enteritis	*Bacillus amiloliquofaciences* H_{57}	[22]
Intestinal barrier, antioxidant capacity, apoptosis and immune response	*Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10	[23]
Performance in broilers	*Bacillus coagulans* and *Lactobacillus* sp.	[24]
Use of nutrients	*Buttiauxella* sp. and *Bacillus* sp.	[25]
Performance, apparent ileal digestibility, blood and excreta characteristics	*Lactobacillus acidophilus; Bacillus subtilis* and *Clostridium butyricum*	[26]
Improving the stress of stocking density, yield	*Lactobacillus acidophilus, L. casei; Enterococos* and B*ifidobacterium termophilus*	[27]
Immune performance and protection	*Lactobacillus acidophilus, L. casei, Enterococos faecium* and *Bifidobacterium bifidum*	[28]
Energy digestibility, performance, disappearance of non-starch polysaccharides	Multiceps *of Bacillus*	[29]
Hatching of fertile eggs	*Lactobacillus acidophilus; Bacillus subtilis* and *Bifidobacterium animalis*	[30]
Microbial profile of cecum and litter	*Bacillus subtilis, Bacillus pumilus* and *Bacillus megaterium* (spores)	[31]
Resistance to heat stress	*Bacillus subtilis*	[32]

Table 1.
Most evaluated parameters in broiler probiotics uses.

microbiota [33]. Other studies, showed that nutritional effects where probiotics act by increasing fiber digestion in birds and enzymatic activity [34]. A competitive inhibiting effect, in which high amounts of Lactobacillus bacteria's produce organic acids that enable low pH in the crop that suppresses the colonization of pathogens in the digestive tract were also observed [21].

Testing different strains *(B. subtilis* DSM 32324, *B. subtilis* DSM 32325, and *B. amyloliquefaciens* DSM 25840*)* in isolation and in combination were showed that the tested strains had different abilities to degrade proteins and carbohydrates and inhibit the growth of *C. perfringens* in vitro. The in vivo results demonstrated that combined strains can act more efficiently than isolated strains on performance parameters as well as reducing mortality in birds challenged by *C. perfringens* [21].

Another study [35] used combined strains of *Bacillus licheniformis* and *Bacillus subtilis*, in order to investigate their effects and found that probiotic supplementation can increase profits if associated with dietary restriction. In addition to the feed efficiency, there was no influence on the carcass yield, the relative weights of the liver, gizzards, proventriculus, small intestine and bursa of fabricius, and the visible fat of the carcass was reduced. The association of probiotics with prebiotics was strongly recommended by these authors.

There is a belief that multiple strains combined with prebiotics have a better effect than isolated strains. FAO reported [20] on the use of probiotics in animals states that the benefit of using more than one strain in the same product still not clearly established.

3. Challenge of replacing antibiotics with probiotics

The recommendation for antibiotics to be replaced by other compounds has been the subject of discussions around the world, mainly after the European Community officially banned its total use in animal nutrition, with the disclosure of Regulation (EC) no. 1831/2003. The main reason for substitution is the occurrence of cross-resistance to drugs used to treat bacterial infections in humans [10]. According to the joint report by the World Health Organization (WHO), Food and Agriculture Organization (FAO) and World Animal Health Organization (OiE), there are still countries that do not yet have control over antibiotics that circulate in animal production (Africa and the Latin America). Many of them lack an organizational structure for control and others only limited their use to promote growth of the animals. Most of the European countries have adopted strict measures to control antibiotics that are circulating throughout the food chain and used in hospitals [9].

Following the recommendations of world health agencies, meat exporting countries, such as Brazil, for example, as the largest meat exporter in the world have been adapting to the demands of international markets. Through the prohibition of antimicrobials commonly used as growth promoters in animal production. Through the normative instructions (IN), the Brazilian Ministry of Agriculture and Livestock has implemented the ban on the following substances as a performance-enhancing zootechnical additive: Olaquindox (IN n°. 11, November 24, 2004), carbadox (IN n°. 35, November 14, 2005), spiramycin and erythromycin (IN n°. 14, May 17, 2012), colistin sulfate (IN n°. 45, November 22, 2016) [36], and the substances bacitracin, tylosin, lincomycin, virginiamycin and tylosin (IN n°. 171, December 13, 2018), [37]. The inhibition is mainly for the purpose of export according to the recipient's rules.

To overcome a challenge of antibiotics concerns for human and animal health, substantial research that investigated alternative increased exponentially. Compounds, such as herbal medicines, [38, 39] prebiotics [40], organic acids [41], symbiotics and probiotics [42] aiming to achieve results like that of antibiotics has been carried out. Economic and environmental impacts may occur with the ban of antibiotics according to [43], implying an increase in production costs of edible noble meat, increase in water consumption and in the production of excreta, as it was observed higher feed consumption and worsening feed conversion when no antibiotics are used. However, the impacts with the frequent use of antibiotics resulting in the selection of resistant strains can be more drastic [44].

No significant differences was observed for the performance characteristics in the period from 1 to 21 days in birds of the group of the zinc bacitracin antibiotic, when compared to the group of a probiotic composed of *Lactobacillus, Bifidobacterium bifidum, Streptococcus thermophilus,* and *E. faecium,* with better weight gain being observed for these two groups in relation to the group without the addition of additives [45].

Studies carried out with the strains *Lactobacillus* spp., *Bifidobacterium bifidum, Streptococcus thermophilus* and *Enterococcus faecium* indicate the efficiency of probiotics against pathogenic microorganisms when compared to conventional antibiotics, being able to reduce colonization by *Salmonella enteritidis* in the intestinal segments [46], without changes in feed consumption and feed conversion [47]. The practices of biosecurity in production combined with the use of probiotics are the way to maintain production efficiency and quality of the final product in the current scenario, [17, 48].

Supplementation with *Bacillus subtilis* probiotic strain reduces infectious agents, such as *Salmonella*, improving the intestinal digestive and absorptive efficiency, in which the probiotic is able to control the population and load of cecal *Salmonella*, being similar to the effect of the antibiotic enrofloxacin [49]. According to WHO (2016), salmonellosis is considered a disease that affects people worldwide, and that has some serotypes resistant to conventional antimicrobials [50].

Corroborating the research carried out by [51] in Brazil, that when assessing the prevalence of several *Salmonella* spp., serotypes in chicken carcasses and live chickens in slaughterhouses, found that 50% of establishments had a prevalence above that recommended by Ministry of Agriculture, Livestock and Supply (MAPA). The same authors evaluated the resistance of *Salmonella* spp. serotypes to two groups of antimicrobials, fluoroquinolones and beta-lactams, and observed that the strains were more resistant to beta-lactam antimicrobials, which demonstrates the risk in the treatment of clinical conditions of salmonellosis in humans.

Another study [52] showed that the use of probiotics, and probiotic combined with 1% garlic powder, was able to promote villi with good width and height in the small intestine and improvements in the performance of birds in the absence of antibiotics, although no improvement in feed intake has been observed. The antimicrobial characteristics of garlic combined with the probiotic reduce the microbial load that in response optimizes the absorption of nutrients in the small intestine.

Although lower feed consumption is observed in some studies, the probiotic allows improvements in other characteristics of greater economic importance such as body weight and feed conversion [53]. Lower feed consumption can be influenced by factors such as heat stress and environments with greater health challenges [54].

4. Promoting growth mechanism by probiotics in broiler

Naturally, the beneficial microbiota that inhabits the intestines of broilers uses a competitive suppression mechanism (as shown in below **Figure 1**) to reduce the multiplication of others that are pathogenic, preventing damage to the mucosa by

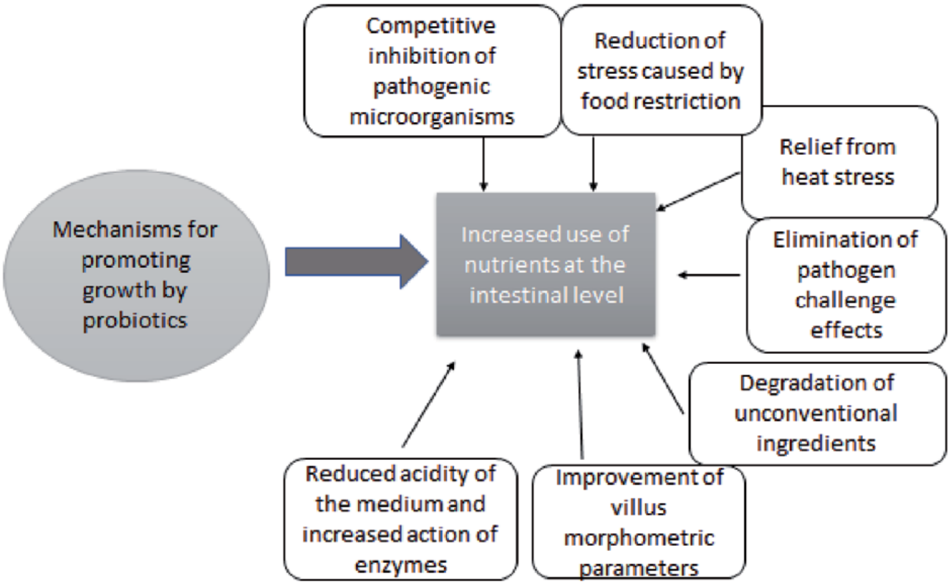

Figure 1.
Possible mechanisms of performance promotion by probiotics.

irritating toxins, controlling enteritis, and guaranteeing protection to the mucosa [5]. Mechanisms are described according to which microorganisms (1) competes for the site of adherence in the mucosa, for better use of nutrients and greater reproduction, in which the one in greater quantity will serve as a barrier that restricts the adherence of others in lesser quantity, reducing possibilities of the latter to multiply; (2) there is the production of organic acids that lead to a reduction in the pH of the medium and the establishment of an acidity that eliminate or decreases all intolerant microbiota, the majority of which are pathogenic; and (3) there is an activation of the immune response by the receptors (toll-like) present in the wall of beneficial bacteria, which, when in contact with the cells of the intestinal mucosa stimulate them to produce immunoglobulins, that will inhibit the multiplication of the pathogenic microbiota [18].

In environments where chickens are subjected to heat stress, probiotics in association with trace minerals, can help maintain or even improve performance parameters, resulting from the increase in the surface area of the intestinal villi [55].

The authors [56] found that the use of probiotics improves blood biochemical parameters, reduced serum uric acid concentration, modified intestinal microstructures and reduced enterobacteria in the ileum and cecum.

Probiotics in combination with other natural additives helps to make better use of the nutrients of alternative ingredients, which for the most part, have anti-nutritional factors, enabling the best use of these for the production of animal feed [57]. [58] concluded that broilers fed 15% of shea butter cake flour added to probiotics, obtained better carcass characteristics and noble cuts when compared to the control diet without probiotics and without flour.

Probiotics act in the restoration of intestinal microflora, decreasing inflammatory processes caused by pathogenic bacteria, with an increase in villus height, and improving zootechnical performance characteristics [59]. This effect was achieved due to competitive exclusion, reduction of enterotoxins and direct antagonism [60]. Other work also show that the use of probiotic improves the body weight and feed conversion of birds, when observing the increase in the levels of glucose and albumin in the blood, which indicates better digestion and absorption of nutrients. The same authors suggest that studies must be carried out to investigate the ideal concentration of probiotic in the feed [61].

The modes of action of probiotics are generally not understood. When summarizing the advantages of using probiotics, one should emphasize their role in protecting animals against pathogens, increasing the immune response, reducing the need for antibiotic-based growth stimulants and high safety of these formulas [19], these factors all contribute to the greater use of nutrients by the bird and, consequently, to a better performance.

5. Limitations on the use of probiotics in broilers

The limitations surrounding the application of probiotics are several, however those inherent in the breeding environment are those described here, among which are summarized below.

5.1 Absence of a pattern in the effect of probiotics

The absence of a common pattern in the effect of probiotics and their probiotic potential is directly connected to particular species, not to the genus or species of a microorganism. Studies with *Bacillus subtilis*, analyzing from performance to biochemistry parameters showed variable results [21, 24, 62–64].

Using Probiotics based on *Bacillus subtilis*, bone growth in broilers under an episode of cyclic heating was induced, which was attributed to the inhibition of bone resorption, resulting from the negative regulation of circulating TNF-a and CTX, [32, 65].

In chickens challenged with *C. perfringens* tested strains of *Bacillus subtilis*, they showed no significant difference in the ability to degrade proteins and carbohydrates and inhibit the growth of *C. perfringens* in vitro, when compared to the control without probiotics, [21]. Already combined with *L. amiloquaficiences*, the results demonstrate the suitability of the combination of multi - strains of Bacillus evaluated as an effective probiotic.

A study by Liang [64], showed that probiotics *(Bacillus subtilis* and *Lactobacillus acidophilus*) in isolation, were not effective in controlling *E. coli* infection in broilers and did not lead to significant weight gain. In addition, 75% of the mortality was obtained in treatments with the application of these probiotics. The combination of these probiotics with flavonoids (Taraxacum) improved indicators of diarrhea [64].

According to assessments made on the use of probiotics in animals by FAO the effects of probiotics seem to result from their interaction with the host. Such interaction is likely to define the mode of action of probiotics, which may be similar in different probiotics, or a specific strain may function through various mechanisms and several strains of probiotics have similar effects on the gastrointestinal microbial population [20].

5.2 Post-marketing handling and conservation

The stability of the probiotic in the storage time is one of the criteria of useful technology established to demand from formulators [66]. However, situations of inadequate handling or conservation after acquisition are more likely to happen and may result in inactivating their effect, and consequent absence the effect now expected, since it is a live microorganism.

The probiotic formulas, depending on the species and age of the host animals, can be administered as a powder, suspension, capsules, pellets, gel or paste [67]. In broiler chickens the most usual form of administration depends on the manufacturer, usually applied via injection (in eggs); via litter; via suspension and also orally.

In oral application conventionally made by means of food or water, the probiotic may be subject to loss of viability in contact with water disinfectants (chlorine) or by interaction with other substances contained in feed. Probiotics are not always effective in reducing microbial load in the same proportion, their effectiveness under commercial conditions is not guaranteed [68].

Eggs injected with probiotics before incubation to verify the effect on their hatchability, had unsatisfactory results, in which no protective effect was obtained [30].

5.3 Existence of internal animal or environmental challenges

Several factors can induce stress in chickens, which results in a reduction of beneficial microorganisms throughout the intestinal tract and their inefficiency in their protective action. The same analogy is used in the administration of the probiotic, in minimum concentration values it is necessary to ensure that they are available until reaching the site of action in the intestinal tract.

With the chicken's intestinal microbiota variable, due to factors such as geographic location, breed, temperature and dietary ingredients, it is very possible that other commensal bacteria can nullify, reduce or amplify the effects that probiotics can have on animal behavior and neurochemical metabolism [69].

The use of combined probiotic *(L. plantarum, Lactobacillus rhamnosus, Enterococcus faecium, Candida pintolepesii, Bifidobacterium bifidum* and *A. oryzae)* had no statistical difference when compared to the control without probiotic, did not affect the performance, internal organs and blood parameters of the chickens challenged with *Clostridium perfringens* [34]. However, in preliminary in vitro studies, this same mixture of strains showed abilities to inhibit the growth of the pathogen.

The mechanism by which the effects of stress or challenge are reversed are associated with the stimulation of the immune system, which in turn activates the nervous system and occurs the restoration of body balance and the fight against the pathogen [46].

5.4 Lack of suitability of the probiotic manufacturer

There seems to be no rule for combining probiotic strains in a formulation, nor an indication of the expected effect of such a mixture to treat a specific problem, which strengthens the use of probiotics as prophylactics rather than as therapeutic. [19, 20, 69, 70].

Information about the formulation is extremely important, especially for commercial broiler production farms or other animals that routinely use probiotics or other prophylactics (live, inactivated, and similar subunits) in their animals without knowing if there is an improving or canceling the effects of each other [69].

Some commercial formulas of probiotics combined, are still sold with indefinite constitution of the strains. A study was carried out with probiotic compounds with undefined strains that were purchased from authorized local stores. That, tested in the treatment of broilers challenged with *Salmonella enteritidis*, the positive effect was only in the first 5 days [46]. There is an assumption that probiotic strains reduce the concentration over time throughout the intestine [70] and, for the best effect, continuous inoculation is necessary to maintain the optimal levels of the probiotic load, capable of competing favorably [18]. Even so, the possibility of dishonesty on the part of the manufacturer is not ruled out. According to the established standards FAO [9, 20] for the manufacture of probiotics, species and strains, as well as minimum viable quantities must be respected and declared.

Studies carried out due to the performance of tests to confirm the information contained in the label of probiotics of several commercial brands, it was found that there were numerous irregularities, highlighting whether (1) existence of strains different from those declared; (2) Encapsulation material not suitable for animal consumption or to maintain viability within the stated period; (3) association of species or strains other than those declared and (4) non-viable microorganisms in packaging within the time limit [71–75].

If there are inconsistencies in the results of probiotic research, information on the safety of a specific microorganism should not be applied to other closely related microorganisms.

6. Risks inherent in the use of probiotics

The absence of clinical side effects is an important benefit of using probiotics. However, some of the species and/or bacterial strains can present risks, they can be responsible for a series of problems to animal, human health and the environment, causing mild reactions or serious and potentially fatal infections [20]. In addition, no probiotic can be considered 100% safe or at zero risk.

Risk reports on the use of probiotics are scarce. However there is a probable risk of transmission of antibiotic resistance (eg, *Enterococcus* genus) to pathogenic microbiota or enterotoxin production (eg, *Bacillus cereus* strain) [19].

In the USA, probiotics and other microorganisms used as food additives must have the status of Generally Regarded as Safe (GRAS), regulated by the FDA. In Europe, the use of the term Qualified Safety Assumption (QPS) has been regulated by EFSA. Crucial criteria for the selection of probiotic microorganisms in animals were jointly established by (WHO), (FAO) and EFSA, where they stand out: Security, functionality and technological utility [19].

Security - stipulates that the origin of the strain to be used as a probiotic must be from the intestine of this animal species; whose antibiotic resistance profile must be non-existent; in addition to the need for a total absence of association with pathogenic cultures.

Functionality- as an important criterion, functionality is about the viability of the strain within the conditions of the intestine, which must be greater; in addition to the efficiency in adherence to epithelial cells, reduced intestinal permeability of the mucosa and proven immunomodulatory effects.

Technological utility - no influence of probiotic microorganisms on the sensory properties of meat or meat products should be observed. Phage resistance is also an important property in this criterion, as well as the guarantee of viability of the microorganism during processing, and good stability in the product and in the storage time.

Current levels of information about probiotics are not sufficient to declare any group of probiotics completely safe without a case-by-case risk assessment. The results of the studies do not allow establishing the existence of a real risk for probiotics, but they also do not guarantee total safety.

7. Final considerations

There is still a long way to go and studies must be done to optimize probiotic formulas in order to guarantee the desired effect. Promising steps were evidenced with the administration combined with other strains of the same or different species and also with other additives such as: phenolic compounds, enzymes, prebiotics and unconventional ingredients.

Generally, the efficacy of probiotics in broilers is evaluated by parameters such as weight gain, feed conversion, and resistance to pathogens, ignoring other aspects such as commercial or natural conditions of the product used, its interaction with feed components and the environment, and their impact on the animal's response. During the use of probiotics, other mechanisms by which these live microbes can affect the host, such as the role in the maturation of the nervous and enteric systems that mediate animal behavior through the gut-brain-microbiota axis, need to be considered.

Even with validated effects of probiotics in several studies, research still shows that the different responses found suffer influences related to environmental factors, specific to the host or the strain, dosages recommended by the manufacturers of the products, types of strains used either alone or in combination, joint use of zootechnical additives, as well as different experimental conditions and greater or lesser health challenge in the broiler breeding phases.

Considering all of the aspects described above, new approaches to checking the effects of probiotics will be possible, and it would be interesting to go beyond the usual comparison that focuses on "treated animals compared to untreated animals" that is observed in most studies to assess effectiveness of probiotics. Both in research and in the application (judged by the supplier market) of probiotics, the role they play as an additive is evidenced, not exactly as a growth-promoting additive, but one that improves, restores, and or installs the optimal conditions for the obtaining the best performance in broilers.

Conflict of interest

No conflict of interest

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Chapter 10

Nutrition and Poultry Coccidiosis: Causes, Consequences and Current Strategies to Modulate the Disease

Luis-Miguel Gómez-Osorio, Jenny-Jovana Chaparro-Gutiérrez and Sara López-Osorio

Abstract

Eimeria spp. are parasites specialized in invade and replicate in the intestine, causing coccidiosis, an enteric disease of major economic importance worldwide. The disease causes losses in production and high morbidity ranging from bloody enteritis, with high mortality, to being subclinical silent but affecting feed intake and efficiency. However, intestinal lesions of the infection vary, depending on the species of coccidia. The most important *Eimeria* species in poultry are: *E. tenella, E. acervulina, E. maxima, E. necatrix, E. mitis, E. praecox* and *E. brunetti*. All those species affect different anatomic sites of the intestine. Thus, they alter the homeostasis of the host reducing nutrient absorption and utilization. Nutritional factors are key players in several steps of the coccidiosis disease. Firstly, as a susceptibility or protection factor, secondly, during the process of infection and pathogenesis, and thirdly, in the recovery and compensatory growth of the bird. Otherwise, coccidiosis also triggers immune response in the intestine. To counter these complicated effects, there are nutritional strategies (including formulation of key amino acids, vitamins, short and medium chain fatty acids, prebiotics, enzymes, among others) that can be utilized to reduce the infection, alleviate the signs, and boost the compensatory growth after infection. This chapter review the impacts of coccidiosis in nutrition and discuss about of strategies to mitigate these risks.

Keywords: *Eimeria* infection, amino acids, digestibility, nutrient absorption, compensatory growth, immune response

1. Introduction

Coccidiosis is a worldwide disease caused by the Apicomplexa protozoa of Genus *Eimeria* [1]. This parasitic disease causes economic losses in poultry industry due to malabsorption, reduced weight gain (explained by a reduced feed intake and as well as nutrient absorption), increased mortality and the use of anticoccidial drugs and vaccines [2, 3] and remains as a major issue for poultry producers in a large number of countries including USA [4], costing for the global poultry industry over 3 billion dollars annually [5, 6]. However, subclinical coccidiosis (Coccidiasis) is more costly to producers than clinical coccidiosis because of impairment on FCR (Feed Conversion Ratio) and decreasing in BWG (Body Weight Gain), slightly resistance of anticoccidials as well as the difficultness in the diagnostic [6, 7]. The infection with *Eimeria* begins when the bird ingests sporulated oocyst, and the sporozoite replication occurs in the intestinal cells generating damage in the mucosa [8]. The infection is normally caused because of mixture of *Eimeria* species. Each species develops in different regions of the intestine and they can cause mild to severe grades of lesions [9]. There are several *Eimeria* species that infect chickens, but the most important are *E. maxima, E. acervulina* and *E. tenella* [3]. The infection process is very fast, from 4 to 7 days, depending on the species [9]. Previous studies reported that a coccidia challenge resulted in 29.5, 24.7, 18.8, and 96.2% reductions in the apparent ileal digestibility of DM, N, starch, and fat, respectively. Interestingly, the negative effects of coccidia challenge on fat digestibility suggested complex mechanisms involved [10]. The strategies to reduce the impact of poultry coccidiosis including the use of anticoccidial drugs, vaccination programs and immunomodulators (e.g., nutritional strategies and functional ingredients including phytobiotics and probiotics).

2. Coccidiosis and nutrition

From the nutritional standpoint, *E. acervulina* and *E. maxima* are the most influential species because of the strategic anatomical location being in duodenum (affecting the release of pancreatic juices) and jejunum intestine (nutrient absorption) respectively. *E. acervulina* and *E. maxima* significantly reduces the water consumption and feed intake simultaneously during acute coccidiosis, however the ratio of water intake to feed intake did not differ between chicks with coccidiosis and healthy birds. Hence, nutritional deficiencies and impaired nutrient absorption can occur following feed and water intake restrictions [11, 12].

Nutritional factors are key players in several steps of the coccidiosis disease. Firstly, as a susceptibility or protection component, secondly, during the process of infection and pathogenesis, and thirdly, in the recovery and compensatory growth of the bird.

Based on that, there are some facts pointing out that the presence of intestinal lesion scores correlates with decreases in average daily gain (ADG), energy consumption, retained energy and feed efficiency. Increments in maintenance is also reported while also seeing increase in excreted energy as well as reduced digestibility in most of the dietary nutrients [10]. The activation of immune response also occurs and requiring use of nutrients that cannot be addressed to the conversion of nutrients into meat, the key goal of broiler production. Therefore, it is paramount to figure out that any level of *Eimeria* infection is causing an evident, but difficult to estimate, loss in performance.

The use of good quality ingredients would aid in nutrients losses during infection and might help to reduce the potential secondary health issues. Nutrient dense diets during infection may be used to counteract performance losses and to enhance compensatory growth phase.

3. Malabsorption of nutrients

A reduction in apparent ileal digestibility of some nutrients including nitrogen, starch and fats in challenged birds with *Eimeria* has been reported [10]. As expected, coccidia challenged reduced starch, fat, and amino acids (AA) digestibility. Of these nutrients, fat digestibility was recognized as the most affected by the level of intestinal lesion [10]. Besides, an estimation of the intestinal damage and its impact on fat and AA digestibility was done. For each unit of impairment in total lesion score, digestibility was decreased by 16% and 3.8% respectively [10].

The first sign seen in birds which are infected with *Eimeria* is loss of pigmentation. In some countries, the coloring of the skin of broilers is an important factor when consumers choose the bird that they want to buy, as they directly associate yellowness or even goldenness with high quality, freshness, and country chickens [13, 14]. The extra cost for to achieve the level of desired pigmentation is high being between 8 to 10 percent of the total feed cost. Carotenoids are fat soluble and a loss of digestible energy specially of fats also affects its absorption in plasma. Besides, it is absorbed through the ciliary epithelium of the midgut and, for this take properly, a process of enzymatic hydrolysis of the xanthophylls, present in the diet in the form of fatty acid esters, must occur. During coccidia challenges, there is a decreasing up to 88% in serum levels of lutein. Some species of *Eimeria* cause desquamation and shortening of the villi of the intestinal mucosa and infects the sites of greatest pigment absorption in the intestine of birds [15].

The optimum pH of gastrointestinal tract is crucial for the action of digestive enzymes. However, coccidiosis infection has been responsible of causing malabsorption of nutrients which is related with the alteration of pH and morphological alteration including flattened villi and elongated [16]. The intestinal content was significantly lower in pH in birds after 5–9 days post infection of *E. acervulina, E. mivati, E. maxima, E. necatrix* and *E. brunetti* than in uninoculated control birds causing the impairment in absorption of nutrients [17]. Consequently, *Eimeria*-induced pH reductions likely impact nutrient digestion and absorption in the intestinal lumen. Also, pancreatic and mucosal brush border enzyme activity is affected at the tissue of infection, whereas brush border enzyme activity may be altered in uninfected intestinal regions compensating for losses in nutrient recovery [18]. Decreased activities of digestive enzymes located on the upper half of the villi, such as disaccharidases, indicate a damaged brush border with a decreased digestive absorptive capacity [16].

4. *Eimeria* infection and protein, amino acids and vitamin dietary levels

Nutritionists and parasitologists have argued for a long time the relation between coccidiosis and dietary protein levels. Early studies showed that chickens fed with high crude protein levels (between 20.5 to 22%) and high-vitamin A and B diets (40% more vitamin A, and about 20% more vitamin B of the requirements respectively) compared with low-protein (between 14.5 to 15.5%) and low-vitamin diet and exposed to equal numbers of *E. tenella* oocysts, had a consistently lower excretion of oocysts after 5 days of exposure and less than half the peak of oocyst shedding on day 7 post infection. Mortality percentage was 5% in the high-protein and high-vitamin A diet, compared to 23% in the low-protein and low-vitamin A diet. However, on the fifth and sixth day, the results were completely the opposite, suggesting that a high amount of protein and vitamin on the feed are overcoming the damage of acute coccidiosis. Nonetheless, during a chronic state of the disease, high levels of dietary proteins, caused an impairment of birds health [19]. As a shortcoming of this study, the effect of protein was not separated from the effects of vitamins.

Britton et al. (1964) showed the interaction between dietary protein levels and coccidiosis varying from 0 to 30% in intervals of 5% [20]. They found a significant reduction of mortality rate and in intestinal lesion score with 0 and 5% of dietary protein levels compared with 10 to 30%. In addition, the chickens fed with high amount of dietary protein showed more signs of coccidiosis such as blood in droppings and lethargy during first week of the challenge.

Furthermore, there is a relationship between the amount of dietary crude protein, trypsin activity and susceptibility to coccidiosis infection. To assess that, an experiment was conducted with chicks fed with diets varying protein levels (5, 20 and 30%) and its impact in intestinal trypsin activity. They found low levels of trypsin in 5% of protein fed compared with 20 and 30% protein [20]. A possible explanation is supported by the fact that when the birds are fed with high dietary protein levels, the production of trypsin and bile salts also increases as well. Thus, trypsin is one of the most important gastrointestinal enzymes for excystation of sporocysts from oocysts [21, 22]. The effect of trypsin intestinal levels and coccidia infection was also evidenced by others studies where chicks pancreatic ducts were ligated and there was no infection after orally challenge with *Eimeria* sporulated oocyst [23, 24].

At this regard, a study was conducted to determine the effect of feeding raw soybean containing 20.3 mg of trypsin inhibitor (TI) per g versus soybean meal of 2.2 TI mg/g in corn-soybean base diets in chickens on the risk to infection with 5 different species of *Eimeria* [25]. The key role of trypsin during excystation step in the coccidia cycle and its effect on the susceptibility of *Eimeria* infection was confirmed.

However, the continuous feeding of raw soybean in corn-soybean base diets also extremely developed weight depression and pancreatic hypertrophy, counteracting the positive effects of raw soybean on coccidiosis [25].

On the contrary, a high level of dietary protein was reported as a protection factor against *E. tenella* and *E. acervulina* infection. In contrast, mortality rate in *E. tenella* and the oocyst shedding in *E. acervulina* increase by feeding a diet with a 24% of crude protein. It has been also showed an improvement in the growth curve of chicks between 8 to 14 days post infection as compared with the first seven days post-infection and suggesting that compensatory growth can occur [26]. It would be appearing that the utilization of the maximum potential of compensatory growth requires highest levels in dietary protein or dense diets although more evidence is needed because most of the studies are only performed only seven days post infection disregarding the upcoming phases of broiler growth.

Adverse alterations of coccidiosis on key anatomical specific areas of the intestine such as the jejunum may affect protein digestibility. A compromised reduction in protein digestibility, using appearance of 14C in the blood of chicks fed labeled protein from cholera algae, was observed during an acute phase infection of *E. necatrix*-infected birds (jejunum) versus non-infected birds. Similarly, protein digestibility was not severe impaired in other species of *Eimeria* including *E. acervulina* and *E. brunetti* during the acute phase suggesting that duodenum, ileum, cecum, and colon were less important in protein digestion and absorption areas than jejunum. During the recovery period, a higher protein and absorption was observed above the absorption rates for uninfected birds [27].

Persia et al. (2006) reported a reduced performance, nitrogen-corrected apparent metabolizable energy (AMEn), and a total tract apparent amino acid digestibility (averaging of 12 percentage units for Thr, Val, Ile, Lys and Arg) with *E. acervulina* infection in birds inoculated with 5.0×10^5 sporulated oocyst in a time and dose response manner which was greater for acute versus chronic infections [28]. Ingredients such as fish meal and prebiotics mitigated the negative effects of coccidiosis lesions on cross bred chicks [28].

Parker et al. (2007) showed a decreased apparent total tract amino acid digestibility (ATTD, 8.4 percentage units) using *Eimeria*-vaccinated broilers challenged with a blend of *Eimeria* species compared with non-infected birds, being the branch chain amino acids (BCAA), and Thr and Cys the most affected [29]. Amerah and Ravindram (2015) showed that ATTD was most impacted for Ala, Cys, Ile, and Thr in broilers inoculated with a blend of sporulated *Eimeria* (1.8×10^5, 6×10^3, and 1.8×10^4 *E. acervulina, E. maxima*, and *E. tenella* oocysts, respectively), showing a reduction of 13.4 percentage units in body weight gain (BWG) for all amino acids [10].

The effect of dietary protein on performance characteristics of vaccinated broilers subjected to clinical coccidiosis challenge has been examined. Lee et al. (2011) assessed the effect of dietary protein on the feed varying from 20 to 24% in coccidia-vaccinated chickens at the first day on broilers and subsequently challenged with different species and concentration of *Eimeria* (*E. acervulina* 6×10^5, *E. maxima* 4×10^5, and *E. tenella* 2×10^5) isolated from the field [30]. At day 21, an improvement in BWG and feed efficiency when increase dietary protein concentration regardless of vaccination status was found. However, vaccinated birds had a statistical trend to impair BWG and feed conversion ratio when was compared with non-vaccinated chicks before inoculation. After the *Eimeria* challenge (day 21 of age) and 0-to-6-day post infection, chicks fed with 24 percent crude protein diet had the highest feed efficiency. In the vaccinated group, birds fed the 24% crude protein diet had lower intestinal lesion scores than birds fed the 20% crude protein diet. No effects on compensatory growth were determined because of the end of trial at 6-day post inoculation [30].

Supplementation of some synthetic amino acids and their role in protection to coccidiosis infection are hypothesized that may overcome lesions in vaccinated birds. In this regard, Mussini et al. (2012) assessed the response of coccidiosis-vaccinated broilers to different levels of dietary glutamine (Glu, 0.5, 0.75, or 1%) during the immunity acquisition phases up to 28 days of age on performance and yield meat [31]. When dietary Glu was increased, BWG also increased concomitantly, regardless of the level of supplementation. On the age of 42 days, this observation was evidenced. Meat yield results did not show any effect with the Glu addition, however, a statistically trend ($P = 0.07$) in breast meat yield was observed. It is tempting to speculate, that Glu might be aiding to avoid muscle protein catabolism as well as supporting gastrointestinal and local immune system in the intestine.

In low protein diets, certain amino acids such as Gly, Ser and Pro have been supplemented by using gelatin as a source of conditionally essential amino acids to test their impact on compensatory growth in *Eimeria*-vaccinated broilers over a full grow-out period of 8 weeks. It was demonstrated that these amino acids improved performance because of their role in maintaining the mucosal barrier integrity [32].

The role of Thr (structural and prevalent amino acid of mucin) during broiler coccidiosis is controversial. Wils-Plotz (2013) showed a positive effect on growth performance when Thr was supplemented at 25% higher than dietary requirement. Nevertheless, Kidd et al. (2003) did not find any interaction between *E. acervulina* challenge and Thr supplementation meaning that broiler Thr dietary requirements are not increased during a mild infection of *E. acervulina* [33].

Arg supplementation has been hypothesized that play a key role on innate and humoral immune response during an Eimeria infection. Similarly, alleviate oxidative stress, improve antioxidant capacity, and attenuate the intestinal mucosa disruption. Thus, it might potentially increasing vaccine effectiveness and/or improve the responsiveness to field infections. An experiment varying different dietary levels of Arg and Vit E on the immune response against *Eimeria* challenge at day 14th of age with a field blend of *Eimeria* oocysts showed that heterophil and monocyte oxidative burst was improved with the concomitant inclusion of Arg and Vit E above the NRC requirements as well as serum levels of IgG and IgM [34]. In a recent publication, the effects of varying levels of Arg was measured in broilers challenged with a mix of *Eimeria* showing a promissory result where the levels ranging from 1.24 to 1.44 improving overall growth, intestinal integrity, and morphology [35].

Rochell et al. (2016) evaluated growth performance, ATTD, and plasma concentrations of amino acids, carotenoids, and α1-acid glycoprotein, an acute-phase reactants, in broilers from hatch to 21 d and inoculated with graded doses of *E. acervulina* oocysts [13]. BWG and feed efficiency dropped linearly when the doses of *E. acervulina* increased. Except for Trp and Gly, ATTD values decreased linearly or quadratically for all amino acids, relative to uninfected animals, by an average of 2.6 percentage units for birds inoculated with 1.0 x 10^6 oocysts.

Methionine (Met) is the first limiting amino acid in corn and soybean meal broiler diets that plays a major role in protein metabolism and has been highlighted as a crucial requirement for the immune system [36] and antioxidant defense system [37].

The impact of three dietary Met levels (0.45%, 0.56% and 0.68%) on alleviation of coccidia negative effects in broilers under various anticoccidial vaccination programs was examined in broilers from 22 to 42 d of age treated or vaccinated against coccidia after inoculation of *E. tenella* (5 x 10^4 sporulated oocyst) [38]. Dietary Met levels from 0.45 to 0.56% and 0.68% improved BWG and feed conversion ratio of broilers medicated against coccidia. However, dietary Met levels did not improve performance in vaccinated birds suggesting that Met levels could be benefiting the growth outcome in medicated chickens regardless on vaccinated chickens.

Two different Met sources and dietary levels of supplementation (non-supplemented, free Met and dipeptide Met) in coccidia challenged birds were examined on performance, gene expression related with immune responsiveness, antioxidant system and amino acid transport in Broiler diets [39]. No interaction between challenge and diet effects was found. However, BWG and feed conversion were improved (12.5 higher and 11.8% lower respectively) when free Met was supplemented compared to the non-supplemented birds. They also reported strong statistical differences comparing non-challenged versus challenged treatments for feed intake, BWG and feed conversion ratio. Coccidia challenge led higher amounts of oxidative substances in the jejunum of chickens 6 d post infection and decreased the gene expression of some amino acid transporters and immune response genes such as peptide transporter 1, toll-like receptor 5, interleukin-2 and occluding. Interferon gamma gene expression was also found increased [39].

5. How would nutrition help coccidiosis?

If producers are using coccidiosis vaccines, special diets could be designed to avoid peaks in oocyst production including the use of Glutamine [31].

The use of good quality ingredients is critical. High digestible feed ingredients where the nutrients are more available, the birds would have more probability to retain nutrients [40]. Also, to know the origin of feedstuffs avoiding the intake of pathogen microorganisms to the flock is crucial. Both microbiology and digestibility quality can aid in nutrients losses during infection and help reduce potential secondary health issues including necrotic enteritis, salmonellosis among others [41, 42].

Low protein diets may decrease the probability of infection but would affect BWG [25]. Thus, decreasing the amount of dietary protein it is not practical strategy. On the contrary, nutrient dense diets during infection may help with performance losses. However, excess of nutrients needs to be avoided on the lumen which may provide a substrate for entero-pathogens (i.e., surplus of protein in the hindgut for *Clostridium perfringens* proliferation) [43, 44].

The use of natural compounds including phytogenics and probiotics and beyond traditional strategies (ionophores and synthetics) have been considered to control coccidiosis challenges in the field or to reduce its severity [45, 46]. Phytobiotics have shown effectiveness against *Eimeria* (*in-vitro* and *in vivo*) and indirect positive outcomes related with boosting immune system and improving microbiota functions [45, 47]. Furthermore, phytobiotics affects fecal oocyst excretion and decrease intestinal lesion score exerting a direct effect on the parasite itself and altering its life cycle in the host [48, 49]. Anticoccidial effects of individual plants, plant extracts, and unidentified commercial plant-derived feed additives have been reviewed elsewhere [45, 49] but many of these studies were conducted using unknown blends and therefore repeatability and further investigation of physiologic pathways is scarce. Saponins by virtue of their surfactant properties have anti protozoal activity and they have membranolytic properties, they complex with cholesterol in protozoal membranes, causing cell lysis [50]. Saponins have shown immunomodulatory effects in broiler chickens challenged with a mix of *Eimeria* as evidenced by lymphocyte outcomes, changes in intestinal structure and alterations in cecal and duodenal inflammatory gene expression [51].

Feeding Probiotics as a replacement of traditional anticoccidials in the diet of broiler chickens might alleviate the impact of the disease and suggesting a coccidiostatic effect against *E. tenella*. One explanation is that probiotics aid to maintain intestinal health and integrity, act as an competitive exclusion principle and decrease the risk of coccidiosis [52]. It was also demonstrated a reduction in oocyst shedding of probiotics compared with Lasalocid. However, the role of feed supplementation of probiotics in performance did not show any improvement when was compared with Lasalocid [53].

An interesting study showed the positive effects of combine essential oils and vitamin D in vaccinated birds with coccidian oocyst of *E. maxima and E. tenella* [54]. An improvement in BWG and decreasing in FCR were observed when the essential oils and Vit D were supplemented compared with the non-vaccinated and non-treated birds. Furthermore, ATTD of dry matter tended to increase with the supplementation as well as an improvement in *Lactobacillus* counts.

6. Conclusions

Although coccidiosis has been the topic of a huge of research over the last decades, is still the major health issue on poultry industry and crucial questions remain answered. There are strategies to reduce or prevent the infection as well as to boost the compensatory growth after infection phase. In antibiotic free systems (ABF) coccidiosis control is requiring multifaceted approach. Nowadays, the strategy to control coccidiosis would not rely just only in coccidia vaccines or anticoccidials. Dietary interventions including protein and amino acids supplementations above the requirements may alleviate performance and immunological impairments in both vaccinated and/or unvaccinated broilers. Nonetheless, it varies according to characteristics of *Eimeria*, type of challenge, health status and environmental issues. Some studies are not considering long term effects such as compensatory growth and vaccines responsiveness during two or three consecutive flocks which may affect the parasite life cycle and immune response status. Nutritional requirements during coccidia infection can be improved by estimating the exact amount of dietary protein and amino acids required for a variety of intestinal functions (such as protein turnover, mucus production, and epithelial cells replacement, among others) and local immune supporting responses such as gut-associated lymphoid tissue. However, this type of information is scarce and further studies are needed to determine the exact role of each nutrient and their impact on performance and health in different production scenarios.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 11

White Striping and Wooden Breast Myopathies in the Poultry Industry: An Overview of Changes in the Skin, Bone Tissue and Intestinal Microbiota and Their Economic Impact

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Abstract

Considerable advances in the poultry industry have been observed in the last sixty years. Consequently, new technological and biological processes have accompanied the development of animals and inputs. With these new possibilities and growth in the sector, there was also the emergence of new paradigms, one of which being the different types of myopathies. In the poultry industry, the breast is one of the noble parts and, therefore, a lot has been studied about the occurrences, alterations and/or different myopathies that alter its quality characteristics. Here we will highlight White striping (WS) and Wooden breast (WB), both investigated more recently due to changes in quality characteristics and considerable losses. The objectives of this review will be to discuss the biochemical parameters of these meats affected by myopathies WS and WB and their consequences on the development of skin, bone and intestinal microbiota lesions; correlate with the impacts of these occurrences to economic losses associated with partial and total condemnations of the carcasses. Another approach is that fast-growing animals have a drop in their quality of life, impacting the well-being of birds since the inflammatory process and excess weight have a direct correlation with dermatitis, corns, arthritis and other comorbidities.

Keywords: quality of poultry meat, broilers, animal welfare, carcasses condemnation

1. Introduction

Approximately 60 years ago, there was a considerable advance in the broiler industry. Consequently, new technological and biological processes have accompanied the development of both animals and inputs. In the last 20 years, there has been an increase in the preference for chicken meat by the general population, the trigger for this event being the affordable price of this protein source, ease and diversity in its preparation, as well as properties associated with the healthiness generated when it comes of white meats. These factors led to an increase in the poultry sector, seeking fast-growing strains, to increase production in a shorter time [1].

With these new possibilities and growth in the sector, there was also the emergence of new paradigms, one of which being the different types of myopathies. In the poultry industry, the breast is one of the parts considered noble and therefore, the occurrences, alterations and/or different myopathies that alter the quality characteristics of the breasts have been studied a lot. The most common are: Pale, Soft and Exudative (PSE), Dry, Firm and Dark (DFD), Deep pectoral myopathy or Green chest, Acid chest, White striping (WS), Wooden breast (WB) and Spaghetti meat (SM). The last three myopathies are the most recent when compared to the others mentioned. Still, there is a need to investigate further, due to changes in quality characteristics and considerable losses for the sector. In addition to permeating hypotheses to better describe what happens, why it occurs and possibly, understand the mechanism to minimize or even suppress them. Several researchers from different countries such as the USA, Italy, Spain, Brazil, Finland, Canada, China and England are looking for these answers. However, until now, little is known about the effects or action of specific proteins and protein groups on these anomalies. The difference between protein content and collagen and protein degradation rate was described in [2]. Although, protease groups are crucial to understanding what can happen with muscle activity.

Although information about the incidence of these myopathies is limited and sometimes contradictory, it is assumed that myopathic chicken breasts appear in all countries where fast-growing hybrids are used, with WS being the most common affecting up to 50% of the breasts of chicken in Italy, France, Spain and Brazil. In the Northeast of Brazil, the proportion of these myopathies is reported between 10% and 20%. In the USA there is an incidence of 98% of birds developing these myopathies, of which 55% were classified as moderate and severe [1].

The trend in the production of Brazilian chicken cuts is to continue increasing, and with that, there is a concern with the quality of this product and with the need to slaughter larger chickens with higher yield and in less time. When all these factors are achieved, a satisfactory result is achieved for the industry and the producer [3]. In a relatively short time (**Table 1**) [4], genetic selection, associated with management, nutrition and other factors, significantly accelerated the development of the muscle tissue of these birds, especially the breast (Pectoralis major), which

Year	Carcass weight (kg)	Age (days)	Breast size (%)
2001	2.207	43	15.8
2007[a]	2.200	36	18.6
2012[a]	2.200	35	21.1
2014[a]	2.200	34	21.5
2017[a]	2.200	34	22.0

[a]Ross 308 Broiler Performance Objectives.

Table 1.
Advance in the production of breast meat (proportional to the carcass size) concerning the commercial Ross 308 line between the years 2001 and 2017 [4].

currently exceeds one-fifth of the total weight of the birds, and certainly represents the most valuable and noble part for the broiler industry.

The results of this project on White striping (WS) and Wooden breast (WB) in poultry will be useful to better understand what happens in these animals, what their impact on meat and carcass as a whole and ensures the slaughtering and consumer industries that will have, even with myopathies, viable products in terms of nutritional, physical–chemical, biochemical and technological aspects.

The birds are staying on the farms for 9 days less and increasing 6.2% in their weight in brisket. This gain is significant for the sector since to keep these animals on the farms, generates costs of food, handling and maintenance of the production system as a whole. In this sense, [5] described that a great ally for the sector's economy is the genetic selection (**Figure 1**), as it brought standardization of broilers concerning body weight, carcass yield and feed conversion, reaching thus, a possibility of slaughtering birds with greater weight in a shorter time. It is known that genetic selection in conjunction with the accelerated growth of these animals does not always achieve positive responses and the incidence of abnormal physiological consequences begins to appear more frequently and is extremely visible and significant in chicken breasts [6].

According to information published by [6], one of the most common changes in the broilers' breasts is deep pectoral myopathy (or green muscle disease) which, according to [7], occurs when oxygenation in the smaller pectoral muscles (commercially known

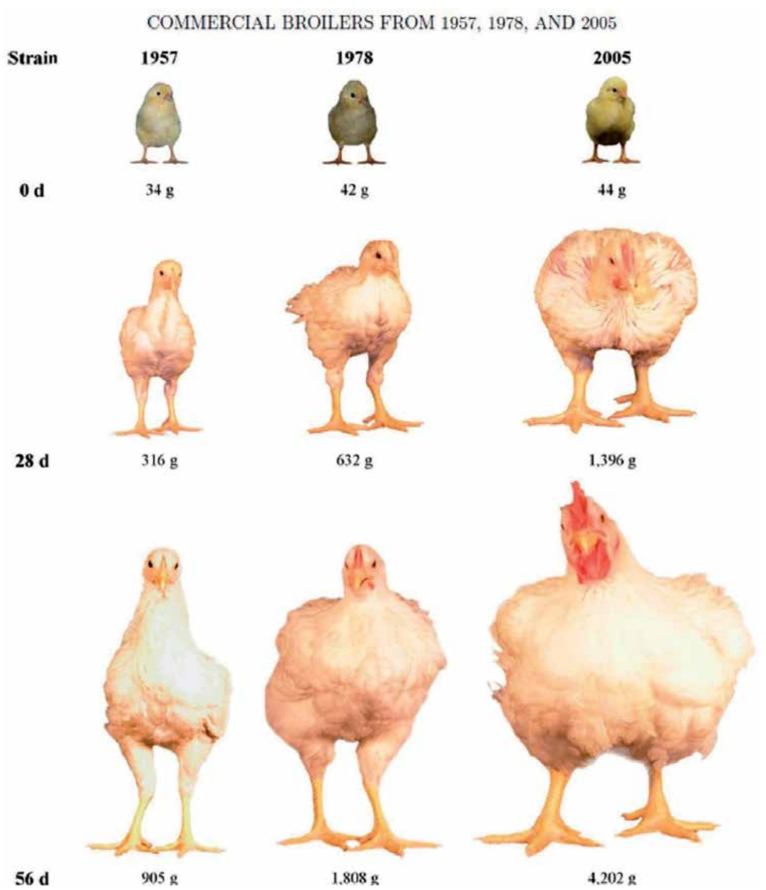

Figure 1.
Growth, efficiency and productivity of commercial chickens from 1957, 1978 and 2005 [6].

as "chest file" ceases or "sassami") with degeneration, necrosis and atrophy. In reference [8] reported that this anomaly can also be caused when blood circulation ceases due to intense muscle exercise, with voluntary movement of the wings where the muscle is unable to expand and ischemic necrosis of the chest muscle occurs.

Therefore, during the deboning process, this muscle is condemned, however, when there is production of whole chicken, it is not possible to identify it, because according to Ordinance N°. 210, of November 10, 1998, the supra-coracoid muscle is exposed only when the carcass follows a more detailed evaluation at the Department of Final Inspection (DIF) or in the boning room [9]. However, in a review published by [6] that mentions research by Pereira et al. [10] found that myopathy can be a technopathy caused by changes in technology and, if possible, adapt the pre-slaughter management to reduce the damage caused, and these can be consumed, because it is not an issue food security, but rather a product quality problem. Reference [11] pointed out that the green color probably came from the transformation of myoglobin in anaerobic conditions and not from inflammation process.

Others anomalies in the chest and small chest file have caused damage to the refrigerators. In [12] observed in Finland that there was an increase in chicken breasts with abnormalities that was characterized in the pectoralis major muscle, with pale and hard external areas with white streaks, in which they caused rejection by the consumer and, consequently, there were economic losses in industries. They did not find a relationship between these anomalies and any antemortem symptoms.

Through electron microscopy analysis, this type of anomaly was called WS (**Figure 2**). Still, concerning anomalies, another occurrence in chicken breasts was investigated by [13] and called WB, however, the breast had a yellowish color and a certain hardness accompanied by inflammatory processes and necrosis. Both anomalies had similar histological characteristics. However, there is no information about the implication of these anomalies in the quality of the products, as well as the training mechanisms.

White streaks of chicken breast called WS (**Figure 2**), according to [8] are related to adipose tissue according to histological and chemical analyzes. While the characteristics of chicken breast with WB are related to the connective tissue that was characterized by muscle hardening [12]. Thus, these two myopathies highlighted in the present proposal, present specific differences, being that WS was characterized by the development of white fibers of the connective tissue and that

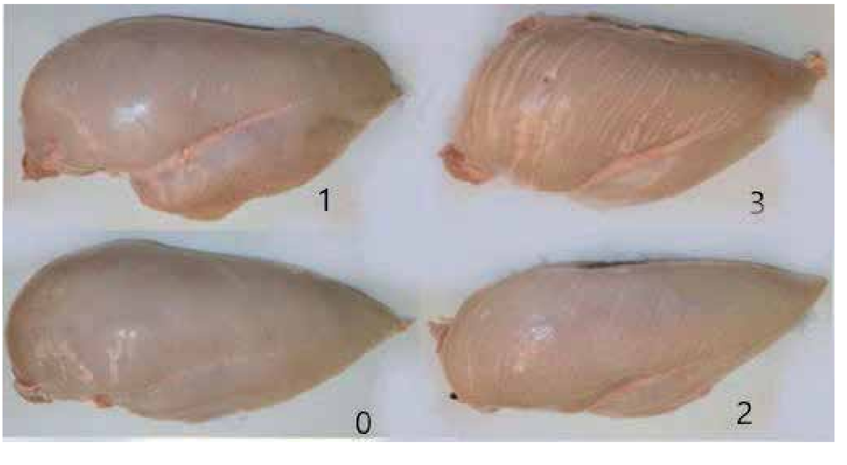

Figure 2.
Breast fillets displaying different degrees of white striping. Score 0 indicates no white striping and score 3 indicates severe white striping [8].

was developed in parallel to the muscle fibers, with the inclusion of adipose decision [14]. While WB according to [15] presented heterogeneity of color, excessive superficial exudate and loss of muscle elasticity.

Published by [16] illustrates in a very creative way how the muscle affected with WB develops pressure resistance. These authors explain the probable etiologies for the development of myopathy and address interesting issues such as nutritional, environmental and genetic aspects.

When or when the animal dies, there is blood circulation failure as a result of bleeding, which causes depletion of oxygen and nutrients. The metabolism then uses the oxygen associated with myoglobin to continue the aerobic process. When O_2 reaches its critical limits, the main metabolic pathway for ATP generation becomes the glycogen reserve. This scenario fully characterized as anaerobiosis, generates lactic acid and reduces the concentration of ATP until it no longer exists in the process. In the sequence, the actin-myosin interactions begin, forcing the muscle to enter a phase of continuous contraction until the muscle enters an irreversible phase contraction known as rigor mortis. Thus, glycogen levels begin to decrease and lactic acid is the product of this metabolism, which accumulates in the muscle fiber, acting as an indicator of the post-mortem glycolysis rate and directly results in a reduction in pH after 24 hours of slaughter. Finally, there is a proteolytic rupture of the muscular structure, which can last up to two weeks, with increased flexibility and tenderness of the meat, this last stage is known as *post rigor* mortis [6, 17].

There are several negative consequences concerning the development of these myopathies directly on the quality of chickens cited by [1], which in turn affects consumer preferences. Therefore, the search for possible solutions to prevent this occurrence is one of the main objectives for food and animal production scientists. Therefore, a prior indication of the pathological pathways of these myopathies is necessary.

2. Economic impact and losses for the poultry industry

A variable portion of chicken breasts affected by the aforementioned myopathies were reported by [1] and may be due to: This author lists in the following sequence, factors that are a consequence of myopathies, being: (a) condemnation/cut (whole breast, carcass); (b) lower yield and value since there will be changes in the water retention capacity [WHC], emulsification and gelation capacity; (c) manual separation in the deboning line to be intensified (addition and training of personnel for classification/sorting - highest cost); and (d) there will be rejection from consumers, since undesirable sensory changes occur in this meat". Consequently, all these factors are responsible for economic loss in the poultry sector.

As there are different forms of myopathies already cataloged, if all of them manifested together, certainly a refrigerator could lose millions in a few hours of slaughter, taking into account that there are systems that work with 350.00 heads of birds slaughtered in a 12-hour period (personal note author). Knowing this, several studies were carried out to try to quantify the percentage and some of the dollar values of the damage that WB and WS can generate.

In a study carried out in the United States, it was reported that more than 50% of the studied squad developed WB [18]. Additionally, study with growing birds on farms under commercial conditions observed that 96.1% developed WB [19]. In 2017, in Italy, [20] observed 474 carcasses and of these, 53.2% developed WB. Regarding values, there are two articles regarding this survey, with Kuttappan et al. [16] indicating annual economic losses for the American market in the range of $ 200 million. For the Brazilian market, there are estimated values of

$ 70,632.00 thousand/day, this being the only work that associated WB with WS to calculate economic losses [21].

The data provided above are reported by different authors, however, the group responsible for preparing this review has worked directly with slaughterhouses in the Southern Region of Brazil and some data are being obtained gradually. Data collected during 30 days of slaughter monitoring during the year 2020 raised the following numbers: of the total and partial convictions of the carcasses as a consequence of dermatoses, myopathies, arthritis, contusion/fracture and contamination, a loss of around $ 162,926.49 occurs a total of 2 million birds slaughtered. This value represents about X% of monthly loss for the sector. If there is an extrapolation that these condemnations can be minimized in the same way as WS my WB myopathies and that they can be associated with the same etiologies, that is, with the rapid growth of these animals, and this equation can be adjusted so that the gain of weight is gradually adaptable to the physiology of these birds, these changes could be minimized and the condemnations, in turn, would gradually decrease, which would generate greater revenue for the slaughterhouses.

Given the great economic loss caused by these myopathies, their characterization and understanding concerning their development, changes in meat quality standards, possible changes in carcasses beside the affected muscles and how these occurrences can affect meat processing, whether or not being associated with a loss of welfare of these animals is necessary information for the sector. The compilation of this information, in a systematic way, could minimize the negative impact of these myopathies, resulting in a higher yield for the sector, as well as an improvement in the quality of the final product that will reach the consumer.

3. Proteins and connective tissue

Protein material is a determinant when it comes to meat. In reference [6] comments that the muscular protein organization and nature is important in the way they are metabolically reversed. The skeletal muscle has three proteic classes based on its solubility; the myofibrillar class is the main one and, as myofibrillar proteins are built in a myofibrillar structure in the striated muscle, presenting a challenge for protein turnover. Analyzing these recent advances in the understanding of this protein system, there are indications that myofibrillar proteins are first hydrolyzed before being degraded and reused. It is still not entirely clear how this dissociation occurs, it is suggested that there may be the release of a group of easily hydrolyzable myofilaments, or it may involve the exchange of myofibrillar proteins in the cell's cytoplasm, or both mechanisms may occur at the same time [22].

In addition to the information mentioned above, on quality parameters for chicken meat, it is necessary to target other lines of research that can provide differentiated responses, or that the responses add up to have a system of information that takes the responses that the industry is looking for. Therefore, research on protein turnover in broilers has been carried out for a considerable time, however much is still not known about the subject. Still referring review of [6], the authors report that the protein turnover in laying hens is associated with the differences between sexual maturity, age, stage and feeding posture.

Genetic-based research with genes related to protein degradation (IGF-1, ampk, anthrogin-1, MURF1 and Cathepsin B) is being developed, however, its expression changes due to changes in protein turnover. These genes have already been sequenced for chickens [23–26]. In an article published in 2017, the authors [27] make interesting comments, some of which are briefly as follows: protein synthesis rates are not different for normal meats or WS, however, protein degradation rates are different between both; increased expression of the MuRF1 and Anthrogin 1 genes are responsible for the highest rate of protein degradation. Despite these sequences, there is still no understanding for these protein changes in birds. This can theoretically lead to greater protein degradation and consequently high catabolic rates, leading to an imbalance in the body of these birds, resulting in the production of proteins that may be deficient, as in the case of collagen for supporting the skin.

In addition to the possibilities involving protein turnover, and as this can influence muscle development, there are still other hypotheses that are being raised to better define the process of meat formation with WS and WB anomalies. One of them is associated with the number of mitochondria present in the tissue, indicating the possibility of a low concentration of these organelles in these muscles [28]. This could explain the need for healing processes in these muscles, which are characterized by muscle striation, indicating the need for deposition of connective tissue.

In addition to this information, studies are addressing the welfare of these birds, since compromising the structure of the carcass as a whole, there may be a direct impairment of their mobility. Reference [29] observed a degree of structural abnormality in all samples of chickens with rapid weight gain. However, there are symptoms in birds that do not affect all individuals affected by WB, however, there may be possible links with the environment [16]. It is not known numerically whether WB or WS affects the behavior, the ability to walk or the welfare of these birds. The hypothesis has been raised that these myopathies would harm mobility and, therefore, have welfare implications for animals.

If there is bibliographic support concerning the mobility of these animals, consequently, this information can be extrapolated to a probable deficiency in the production of connective tissue, since there must be a deviation of nutrients in the metabolism of these animals, to supply the inflammatory processes. Muscle and joint. According to [30], the main histological lesions of the WB muscle, which consist of chronic myodegeneration with regeneration and interstitial edema, accumulation of loose connective tissue or fibrosis and replacement of severely degenerated muscle fibers with connective tissue with excessive fibrosis.

As the connective tissue is crucial for the development of the entire system, as well as muscles, skin and bones, there is a need for a balanced distribution of nutrients throughout the animal's body. In a study published by [31] there is a report on the difference in the organization of collagen, which may be due to the expression of proteoglycan decorin in the extracellular matrix. Decorina is a regulator of collagen crosslinking and is expressed in levels significantly in strains affected by WB, which would lead to tightly compressed collagen fibers due to the high levels of crosslinking of this protein. Besides, the expression of muscle-specific transcriptional regulatory factors for proliferation and differentiation of muscle cells that lead to muscle regeneration in response to muscle damage was significantly elevated for these myopathies. The lack of decorin has been mapped as a destabilizer of the collagen structure due to abnormal collagen crosslinking, leading to fragility of the skin, caused by an abnormal fibrillar network [32].

3.1 Collagen, bone tissue and fractures

The information mentioned above in this brief literature review shows an overview of the problem, however, through reports from slaughterhouses, in the day-to-day work of the Research Group that is developing this proposal, there is evidence that the animals that develop WS or WB are more likely to have problems associated with the quality of their skin. It has been observed in the field and in the slaughter plants that these animals have the most fragile skin, with lesions that occur both within the farms and during the plucking process, there may be a greater correlation of bone fractures with these anomalies and consequently a considerable economic loss for the sector, since the carcasses may have partial or total condemnation.

Research developed by [33] related WB myopathy with problems associated with the formation of bone tissue. These authors evolve an interesting line of reasoning based on the development of bone marrow-forming cells and how the increase in adipocytes can influence this process. As a result, they obtained positive correlations, and the levels of calcium and phosphorus in the bone matrix of animals that develop WB are lower when compared to those not affected by myopathy.

A study by [34] that analyzed the efficiency of magnesium in the control of oxidative processes in birds, as well as its correlation with the decreased incidence of development of WS and WB myopathies, concluded that magnesia supplementation protected the tissue against protein oxidation and that it reduced the incidence of WS and WB myopathies to almost half the occurrence in fed animals supplemented with this mineral. In the same study, these authors report that even Calcio and Magnesium use unusual mechanisms of divalent ions for their absorption, one does not harm the absorption of the other, and the same occurred for Phosphorus. The results were innovative since magnesium did not interfere with the action of other minerals, a positive factor, since this benefits the bone matrix, so supplementation with magnesium in the feed of broilers can be a promising alternative as a supplement to mitigate the development of WS and WB myopathies.

4. Microbiota

Another parallel approach to be considered is the intestinal microbiota of birds, as this is an innovative aspect of the proposal since recent studies have verified the importance of the intestinal microbiota in animal performance, health and well-being [35–37]. There is still a close relationship between the diet, microbiota and bioactive compounds that may be present or that are used commercially in poultry feed. Studies that address the interaction of the microbiota are still limited and are at the frontier of knowledge under the paradigm of sustainable poultry production, prioritizing animal welfare.

The microbiota is recognized as the "fifth organ" and the literature suggests that the microbiome plays a crucial role in signal processing and interaction with the environment [38]. The composition of the bacterial microbiota is affected by the bacteria present in the intestine and by the natural microorganisms in the environment [39]. Chicks born in natural conditions receive the microbiota from adults, mainly from the mother. Industrial poultry farming has altered this condition, preventing the chick from coming into contact with the mother, which leads to a delay in the development of the protective intestinal microbiota [40, 41]. The balance of the microbiota can be affected by several factors, both endogenous and exogenous. Poor hygienic-sanitary conditions, stress, food, intoxication and illness, can trigger the increase in bacterial proliferation that can compete for nutrients. They can also determine inflammatory processes, which leads to thickening of the intestinal wall, which will reduce absorption, increase the excretion of metabolites and toxins that trigger enteritis and decrease the transit time of the digesta. Besides, it can increase the turnover of epithelial cells, which allow bacterial and endotoxin translocation to other organs, leading to septicemia [42]. According to [43] it is important to understand and have control over the possible changes in the intestinal microbiota to adapt the management and to include in a rational way additives that can alter and regulate the microbial ecology, to improve zootechnical performance and reduce some effects of stress or the harm of diseases.

In adult birds, when the microbiota is established, it may contain 400 to 500 microbial species [44]. With variations in the amount and types of microorganisms that may be attached to the epithelium or free in the lumen. When free, they may have an accelerated multiplicative capacity, minimizing loss through peristalsis, and may be associated with other bacteria that are linked to the mucosa. These variations mean that, in general, the small intestine is colonized by facultative microaerophilic bacteria, with their respective representation (in percentage) in the microbiota, which is: *Lactobacillus* (70%), *Clostridiaceae* (11%), *Streptococcus* (6.5%), *Enterococcus* (6.5%). The cecum, on the other hand, has mandatory anaerobic bacteria such as *Clostridiaceae* (65%), *Fusobacterium* (14%), *Bacteroides* (5%), and is also permeated by facultative microaerophilic bacteria such as *Lactobacillus* (8%) and *Streptococcus* and *Enterococcus* [45, 46].

A study by [47] who investigated the microbiota of WB and normal birds to understand the differential expression of plasma metabolites, obtained different results between groups, with non-myopathic broilers produced more heat, with higher body protein content, validated by the higher protein: fat ratio. Lower protein content in myopathic birds was verified, due to the probable high myiodegeneration, as observed by the high expression of 3-methylhistidine in plasma. In this work, the authors also reported that there was a predominance of unclassified Lactobacillus in birds with myopathy; while the species, L. acidipiscis was the predominant bacterium for non-myopathic broilers. The differentially significant metabolites identified in the plasma metabolome between the two groups were homocysteine, cyclic GMP, trimethylamine N-oxide, tyramine, carnitine and acetylcarnitine, all associated with the cardiovascular system. The results of this work suggest that more research on broilers should be carried out with a focus on tissue vascularization.

As WS and WB are proven to be inflammatory processes that permeate the entire carcass, it is possible to raise the hypothesis that alteration in the microbiota may be determinant for birds to be predisposed to develop these anomalies, consequently, there may be changes in the absorption of limiting amino acids or even essential for the synthesis of important proteins such as collagen and other proteins that can provide resistance to blood vessels, bones and skin.

5. Animal welfare: possible changes due to the occurrence of wooden breast and white striping

Brazilian poultry stands out in the international meat market, and according to data from the Brazilian Association of Animal Protein (ABPA 2018), Brazil was in 2nd place in the world production of chicken meat (2017) with 13.05 million tons, second only to the United States, which produced 18.6 million tons. As for exports, Brazil occupies 1st place, exporting approximately 4.3 million tons [48].

Poultry intended for meat production must be assisted to comply with specific conformities for farms, such as maximum animal density, minimum lighting intensity, air quality, water and food availability, among others. Also, meat mortality and inspection data are considered to establish maximum values of stocking density. Dermatitis, parasitic infections and systemic diseases should also be measured to identify signs of poor well-being [49].

Some factors that have directly influenced this growth system are: improvement of lines and inputs, automation of process systems, sanitary conditions for the creation of controlled birds, integrated production system, among others [50].

There is a projection of a 46.4% increase in chicken meat production by 2023 Simultaneously, there is a growing demand for information on ethical aspects of animal production. In a study conducted between the states of Paraná, Santa Catarina and Rio Grande do Sul, evaluating the condemnation of birds, it was observed that injuries were the main cause of condemnation in 2015, followed by dermatoses. These same authors report that the third highest incidence of condensation is associated with dorsal skull myopathy [49]. In the State of Paraná, which slaughter more than the others in absolute numbers, the percentage of convictions increases. The progressive increase in rates of injury conviction, inadequate bleeding, arthritis and aerosaculitis can indicate important aspects of well-being.

The increase in convictions caused by arthritis may indicate that the industry has undergone significant changes in recent years, which result in a negative impact on the welfare of birds [51]. This information goes from other information obtained with the weight gain of these animals since birds are gaining weight very quickly. These same authors indicate that genetics may be the main cause of skeletal disorders in fast-growing breeds, causing a lack of activity in the birds, which because they are very heavy, aggravates the problem [52, 53]. The high stocking density is also related to the reduction of air quality, increased thermal stress and increased transmission of infectious diseases.

A study by [30] observed that for birds with WB there were changes in the birds' behavior since they associated this difference with the change in the way of walking of these animals. Which may suggest possible effects associated with loss in the welfare of these birds. Another study by [16] have already reported a possible change in the growth conditions of these birds, directly affecting their welfare.

Approach to the welfare of these birds is essential, as it compromises the structure of the carcass as a whole and can directly compromise its mobility. In reference [29] it was observed a degree of structural abnormality in all samples of chickens with rapid weight gain. However, in another study by [16] reported that there are symptoms in birds that did not affect all individuals affected by WB, however, there may be possible links with the environment. However, there is still no quantitative information on whether WS or WB affects the behavior, ability to walk or the welfare of these birds. There is a chance that these myopathies would harm mobility and, therefore, have welfare implications for animals. With this information, it would be interesting to study a variable that can correlate the stress level of birds with the myopathies associated with this proposal, with cortisol being an excellent marker for this purpose.

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Conflict of interest

The authors declare that there is not any conflict of interest.

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Chapter 12

The Effects of Heat Stress on Production, Reproduction, Health in Chicken and Its Dietary Amelioration

Mathew Gitau Gicheha

Abstract

Farm profitability is the key driver of most livestock enterprises. The productivity and profitability are driven by genetic potential of the animals and the ability to express the superiority in the production environment. In an ideal situation, an animal should produce maximally as dictated by the genetic potential. It is noteworthy that the environment in which an animal lives in impacts on its ability to expose its genetic potential. Studies have shown that it is rarely feasible to provide animals with ideal conditions to express their full genetic potential. The environment in which animals are reared is characterised by many factors that interact in ways that result in different performance even in animals of similar genetic makeup. For instance, thermal environment is critical in poultry production as it affects both the production and reproduction in different ways. The thermal environment affects chicken differently depending on the stage of growth or production phase. This environment has been impacted by the climate change and subsequent increase in climatic variability resulting in thermal challenges in naturally produced chicken thus altering production and reproduction. This implies that there is need to consider thermal resource in the routine poultry management practices. This would result to design of poultry production systems responsive to the thermal environments more so in the light of climate change and the subsequent increase in climatic variability. This chapter explores the impact of heat stress on chicken production, reproduction, health and its dietary amelioration.

Keywords: Heat stress, Chicken, Production, Reproduction, Health

1. Introduction

Climate has been identified as a factor that has direct and indirect effects on animals via the animal's environment [1]. Farmers will bear direct costs of climate change characterised by reduction in poultry yields and indirect costs of adaptation [2]. This implies heat stress is contributing to low production while simultaneously increasing the production costs. This leads to reduction in poultry enterprises profitability and is a threat to the survival of the sector. Notably, all domestic livestock are homoeothermic which means that they are continuously attempting to maintain their body temperatures within the most suitable range for optimal biological activities. However, poultry are more susceptible to heat stress because they can only tolerate a narrow temperature ranges [3]. Achieving a state of thermal stability without expending too much nutrients would result in high production in systems in where nutritional, health and general animal welfare environments are maintained at optimal states. Heat stress occurs when there is a negative balance between the amount of heat energy produced by the animal and the net amount of heat energy flowing from the animal to the environment [4].

In order to maintain a constant body temperature poultry must preserve a thermal balance between their heat production and gain from the environment. This is achieved physiologically through metabolic and physical means [5]. The metabolic heat production depends on the basal heat production for maintenance of essential body processes [6]. The imbalance in heat production in birds is attributed to various environmental factors such as thermal irradiation, sunlight, air humidity and animal characteristics such as metabolic rate, thermoregulatory mechanisms and species [7].

Whenever ambient temperatures increase beyond the thermo-neutral zone, mechanisms to dissipate heat are triggered (increased respiration and heart rate), and as a result, maintenance requirements increase [8]. Similarly, maintenance requirements also increase during cold weather as the animal needs to generate heat in order to maintain body temperature [6]. The heat production varies and corresponds to the nature of activities the animal is exposed to. Process such as growth, reproduction and health status results to an increase in metabolism and subsequently heat production. Animals are able to respond to decreases and increases in metabolic, digestive and nutrient metabolism, muscular, increased metabolism heats. However, an animal has to maintain basal heat production at constancy since essential or vital body processes must be maintained [5]. There are a number of body heat loss methods [9]. The most important one is evaporation. This method depends on the ambient air temperature, the amount of available moisture, the evaporative surface area, the humidity of the air surrounding the animal and the degree of air movement [7]. The amount of available moisture depends on the quantity of sweat produced by the animal while the area of evaporating surface depends on the surface area of the animal and the size of the lungs, with considerable evaporation being achieved by panting [5].

Loss of heat by livestock through the movement of heat from one object to the other at higher temperature (conduction) is limited. Heat is transferred from the body through conduction at relatively low temperature [9]. Loss of heat from the surface (convection heat loss) increases when a cool breeze blows on the animal and increased air movement. Research has shown that the loss of temperature by convection occurs when heat by from the comb, wattles, face, legs, toes, neck, body and wings is lost to the surrounding air as air circulates inside the poultry house [10]. The cool breeze and increases in air movement significantly increases evaporative heat loss. Livestock houses in the tropics should always be constructed in ways that encourage maximal air movement on and around the animal. This is achievable by ensuring that the animal houses are well ventilated.

The documented normal body temperatures of chicken ranges between 41 and 42°C, while the chicken thermal comfort zone falls within 18–21°C. Study by Wasti et al. [11] showed that temperatures above 25°C results to heat stress. When the balance between body heat production and loss is not maintained then the birds are subjected to heat stress. The balancing occurs in an environment charactersied by interactions of many factors key among them being high environmental temperatures, radiant heat, airspeed and humidity among others. When the ambient temperatures are high, chicken use various physiological and physical mechanisms to maintain a thermal comfort zone [9]. The birds use body energy reserves to maintain a thermal comfort zone. This occurs at the expense of production and growth. This implies that heat stress negatively impacts the productivity and profitability of poultry enterprises. Broiler meat and eggs lose their quality under high temperatures resulting to further postharvest losses.

2. Heat regulation in poultry

Birds do not have sweat glands and rely on evaporative cooling which is aided by panting to regulate their body temperatures. It has been documented that due to lack of sweat glands in birds, most of the heat loss occurs through respiration [5]. Birds have an extensive air-sac system connected with their lungs which is important in heat regulatory function [5]. In the tropical environments, it has been observed that poultry frequently hold their wings slightly separately when standing to allow air flow through while at the same time extending the heat loss surface area [12]. The birds adopt an extended position when lying down to further increase the surface area. Collectively, these behavioural responses maximise on the surface area of the body in contact with the external environment for heat dissipation [12]. Studies have shown that when chicken are exposed to heat stressful conditions, they spend less time moving and more time resting in the shades or cool places [13]. Birds also manage heat by increasing or decreasing feed and water intake [14]. When the temperatures are high, birds tend to reduce their feed intake while increasing their water intake. The reverse holds when the temperatures are low.

Generally, chicks from many poultry species are not able to efficiently regulate body temperature during early and post hatching period [15]. However, at an early age, the chicks should be provided with right temperatures in the post-hatching stage without which they get chilled and die almost immediately. This underlines the importance of good brooding heat management by provide adequate heat (artificially) for the young chicks. The temperatures should be set at around 35°C in the first week after hatching, with a 3°C weekly reduction until normal ambient temperature of about 23°C - 26°C is attained. Lin et al. [16] proposed that the ideal temperature range for neonatal chicks should be set at between 32 and 35° C. It is important to maintain the right chicks' temperature range when transporting them from hatcheries to avoid overheating or chilling which reduces their post hatch-survival rate [17]. Abdelazeem [18] reported that, high ambient temperatures reduce the chicks' growth rate and feed intake. There is general sluggishness in chicks when temperatures are high [17]. This tends to reduce the feeding time and results in reduced feed intake and consequent poor performance in growth. This problem can be managed by formulating high nutrients density chick feeds which would ensure a good supply of nutrients in the face of low feed intake [19].

It has been observed that free range birds maintain thermal neutrality by hiding under shades during the hot hours of the day while resuming the scavenging in the cool hours of the day [13]. The pattern has also been noted with housed birds which stop feeding during the hot hours of the day and resume feeding in the cool afternoon. This is important for poultry producers to note so as to synchronise their feeding strategies to the behaviour in areas characterised by high ambient temperatures.

3. Effect of environment on poultry nutrition and production

Various environmental factors including temperature, light, rainfall, humidity and altitude collectively impact on an animal's nutrition [14]. The respective factors can impact on the intake singly or cumulatively. There is an inverse relationship between energy requirements and effective temperature which subsequently affects feed intake mechanisms related to circulating levels of blood components [20, 21]. It has also been documented that high temperatures above the critical thresholds leads to reduced feed intake, lower feed conversion efficiency and lower body weight [19]. It has been observed that heat from an external source or from specific dynamic action of feed has effect on the rostral cooling centre of the hypothalamus which results to a stimulation of the medial satiety centre which in turn inhibit the lateral appetite centre [16]. This results to a decrease in feed intake and subsequent lower the production and/or reduced reproduction efficiency. Broiler birds when exposed to high solar radiation are coupled with depression of chemical composition and meat quality [22].

Birds experiencing heat stress usually pant with their mouths open, elevated wings and have higher tendencies of squatting near the ground [17]. It has been reported that laying flock when exposed to high temperatures shows disturbance in acid -base balance in the blood as a result of hyperventilation, there is excessive loss of CO^2 from their lungs due to gasping [20]. The lowered amount of CO^2 in blood leads to a rise in blood PH causing calcium ions to drop in blood that would have been utilised by the shell gland resulting to poor egg quality [23]. It is noteworthy that increased panting in poultry is a sign of heat stress. Other noticeable indicators of heat stress include elevated respiratory rate, and restlessness which lead to increased loss of body fluids and therefore dehydration [20]. The birds also tend to drink more water in order to cool their bodies while feed intake reduces drastically. These adversely affect the efficiency of meat production and the meat quality especially in the exotic birds not adapted to the tropics. Zhang et al. [24] demonstrated that, when broiler chickens were exposed to high temperature during the growing phase, it resulted to poor meat characteristics and loss of quality. Ranjan et al. [25] observed that when broiler birds are exposed to high ambient temperatures during the growth phase tended to result in poor meat characteristics as well as loss of storage quality [22]. Heat stress reduced the proportion of breast meat while increasing the proportion of fat in the carcass. Zeferino et al. [26] further noted that when broilers were exposed to high ambient temperatures during the finishing phase, the carcass lost quality characters related to consumer preferences such as colour, tenderness and shelf life due to increased meat PH. High altitude areas ranging from 2,900–3,900 m are characterised by hypoxia, lower air pressure and lower ambient temperature compared with the lower altitude areas. This in turn affects nutrients partitioning which has impact on productivity and profitability. More nutrients are shifted to deal with the cold and the low oxygen levels which subsequently affects nutrients digestibility and allocation of the nutrients to various biological processes [26]. In all cases, production and reproduction performances are affected negatively.

It is evident that temperature primarily affects production of poultry meat and eggs through increased or decreased feed intake. Various authors such as [20, 21] reported that there was notable reduction in egg production in hot environments due to decrease in feed intake, decreased digestibility of different diet components and reduced uptake of available nutrients [19, 27]. Generally, feed intake starts to decline when chickens are kept in an environment characterised by temperatures above 27°C. At 35°C there is a significant decrease in feed intake. Besides, studies have shown that the reduction in intake is accompanied by decrease in feed conversion ratio. Optimal nutrient intake and utilisation is a key factor in weight gain and egg production which are the key drivers of poultry enterprise productivity and profitability. Any factor that affect the intake is important to producers whose objective is production optimisation and profit maximisation. In a scenario where the intake is negatively impacted by high ambient temperatures, producers can counter the reduction in feed intake by formulating high density diets. Besides, feed management can be accomplished through housing chicken in well ventilated pens especially in areas characterised by high ambient temperatures [15]. Poultry production systems in environments characterised by high ambient temperatures have reported steep rise in culled birds resulting from reduced productivity and death emanating from heat stroke. This loss makes such systems unproductive and unprofitable.

The high temperatures further cause a decrease in the egg shells thickness and weight [28] in laying hens. This leads to increased egg breakage during the storage and transportation. Generally, egg quality during storage decreases gradually with increasing temperatures [29]. Low temperatures have no impact on egg shell thickness while low temperatures have been shown to increase feed conversion ratio.

Climatic stress in laying hens leads to a reduction both egg production and quality [30]. The notable decrease in egg production can be explained by the imbalance between calcium-oestrogen and the reduced Haugh Unit of the albumen detected in birds subjected to heat stress [31]. This leads to a reduced yolk size, albumen consistency and normal calcium deposits on the egg shells. Birds tend to spend more time resting rather than eating which negatively impact on the available nutrients to satisfy production and reproduction processes and outcomes [32]. Climatic stressors trigger the behavioural, physiological and immunological responses which have detrimental consequences on production and quality. Heat stress shifts more energy to maintenance and acclimatisation functions at the expense of growth thus resulting to decreased body weight gain when subjected to heat stress. Lara and Rostagno [31] noted that the negative effects on production and quality emanate from the decreased feed intake, reduced feed digestibility, low plasma protein and calcium levels. Oguntunji and Olufemi [30] observed that egg sizes which affects their market value decreases with a decrease in feed intake. Scavenging poultry is affected more as the birds spend more time under shades when they should be harvesting nutrients necessary in production and reproduction [33].

It is noteworthy that exposure of day-old chicks to high ambient temperatures during transportation, which has been identified as one of the leading causes of chicks' mortality, leads to exhaustion and death [15]. This means there are less birds available for production and reproduction. Day old chicks should be transported in well ventilated trucks and in equipment that ensure air circulation while at the same time avoiding overcrowding. Besides the need to transport the chicks in appropriate temperature range, high temperatures during transportation of broilers from the farm to the slaughter and processing facilities have been shown to impact the quality meat negatively [34].

4. Heat stress and reproduction

Studies have shown that high ambient temperatures, outside the thermal-neutral zone results to negative effects on chicken biological processes. The optimal temperature ranges between 12°C -26°C. Research findings presented in Ebeid et al. [35] suggest that reproduction of the animal is highly affected with high temperatures. The study shows that when white leghorn hens are subjected to high temperatures there is a decline in reproductive activity leading to reproductive failure and poor egg quality.

Fouad et al. [34] further showed that increased environmental temperatures affect all stages of semen production. Slight elevation of the environmental temperature during the early phase of semen production has been shown to stimulate testicular growth which in turn promotes increased quality and quantity of semen. However, high temperatures in mid and late semen production result in decreased the seminiferous epithelial cell differentiation which results a decrease in semen concentration and volume. This has negative impact on the cock's fertility and flock reproduction in general [34]. The work by Karaca et al. [36] confirmed that heat stress resulted to decreased semen quality.

Findings by Nidamanuri et al. [37] showed that the function of the hypothalamus and pituitary gland is negatively affected by heat stress. Results from a study [38] using leghorns chicken exposed to heat stress depicted a decline in the production of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) which are associated with increased levels of prolactin. Rozenboim et al. [39] observes that the resulting low GnRH, LH and FH (follicle stimulating hormone causes regression of the ovary which reduces the capacity of the theca cells resulting in impaired steroidogenesis. Wasti et al. [11] indicated the levels of gonadotropin-releasing hormone, plasma progesterone, testerone and estradiol hormones is impaired in heat stressed birds which leads to a general reduction in reproductive efficency. The reduced effciency results in lost reproduction and subsequently poultry flocks productivity and profitability. It is further documented that eggs obtained from hens subjected to high temperatures have lower hatchability. High temperatures cause a decrease in granulosa cells responsiveness which results in the disruption of the hormones that are crucial in ovulation. Studies by Ayo et al. [40] and King et al. [41] involved collection of semen during summer to test the effects of heat on the characteristics of spermatozoa and found an increase in deformities including bent heads, cytoplasmic droplets and cut mid-piece. Ayo et al. [40] further demonstrated that semen collected during the cooler periods (especially in the morning) of the day resulted in higher rate of conception when used in artificial insemination.

The endocrine system and the environmental cues are the key drivers of reproductive patterns of an animal [42, 43]. There are various environmental factors that influence reproduction including the day length (more pronounced in temperate regions), temperature, rainfall patterns (seasons), human management practices such as feed and feeding management system, animal population interactions and socialisation, health and nutritional status of the individual among other factors [44]. Taberlet et al. [42] observed that significant alteration of one and/or a combination of the factors could partly or completely disrupt the reproduction function. The environmental factors that influence reproductive processes do so ultimately or proximately which is based on the time at which they affect the breeding activity. The ultimate influencers are more significant as they relate to the effects on long term basis. Food availability is the most important ultimate environmental factor that affects breeding due to the need to synchronise animal feed demand and supply. Ambient temperature is an important proximate environmental factor.

5. The effect of heat stress on response to diseases

The health of a flock has a direct relationship with poultry enterprise productivity and profitability. The costs and lost production associated with unhealthy flock leads to enterprise losses and at times to the collapse of the venture. There are a number of risk factors associated with the health of a flock including the climatic conditions of the area in which the production occurs [1]. Temperature is an important climatic factor that affects the bird's ability to fight off an infection. In order to resist or fight off an infection sufficient and high-quality nutrient are required [15]. The bird should also have minimal heat stress since there would be a competition for nutrients between fighting the infection and search for heat balance in the body. High temperatures negatively affect both the nutrients intake and the chicken thermal neutrality thus exposing the birds to diseases [45].

High temperatures are commonly encountered in the tropics for most of the year and during summer in temperate regions. In sub-optimally managed flocks, the temperatures may rise above the thermal neutral zones thus stressing the birds and exposing them to diseases conditions [11]. This can be counteracted by housing the birds in well ventilated pens, provision of high-density nutrients diets and high level of diseases prevention technologies.

Heat stress compromises absorption of nutrients, integrity of gut wall and the immune system in birds [46, 47]. In addition, the weight of the liver reduced along with reducing levels of antibodies [48]. Another research found that the reduction of antibodies was could be the sole reason for increased incidences of diseases like infectious bursal disease virus (IBDV), Newcastle disease virus (NDV) and infectious bronchitis disease virus (IBDV) during heat stress in poultry birds [49]. High humidity alters the homeothermy in poultry birds and induces the growth of disease causing agents like viruses, fungi and bacteria [45]. Moreover, during hot conditions, incidence of many bacterial diseases such as salmonellosis, coccidiosis and *E. coli* infection increased [45]. It is also documented that broilers that were exposed to heat stress showed decreased immunity and became more susceptible to pathogens such as coccidia that induces necrotic enteritis [50]. Heat stress resulted in reduction of the relative weight of the thymus, Bursa of fabricius and the spleen which also suffered oxidative damage in exposed flock [34]. The decrease in the weight of the organs implied that the affected individuals suffered reduction in the production of antibodies. This results in higher exposure to infectious diseases in heat stressed birds. Wasti et al. [11] identified a spike in the prevalence of infectious and contagious diseases in poultry in summer seasons in the tropics which they related to the higher incidences of heat stress in poultry flocks.

6. Management of heat stress in poultry flocks

The primary objective of managing heat stress is to free up nutrients that would have otherwise gone into establishing and maintaining thermal neutrality in animals to be used in growth, maintenance of good health status, production and reproduction. This in turn lead to increased animal productivity and animal production enterprise profitability. The harmful effects of heat stress due to high temperatures are evident and therefore, it is necessary to adopt appropriate strategies to minimise the impacts of high temperatures and enhance production of eggs and chicken meat to meet the growing demand for poultry products [16]. Heat stress in poultry can be reduced by a multi-approaches strategy including modification of the surrounding environment (housing designs, ventilation systems, provision of shaded areas), nutritional management, stocking density management [51] and selection for heat tolerance genes [52, 53]. The approaches can be oriented towards general farm management or dietary manipulation.

6.1 Farm management

Designing well ventilated poultry house is the first step in the management of heat stress [15]. This can be achieved by installation of efficient air circulation systems in areas characterised by long periods of high ambient temperature. Butcher and Miles [54] notes that this not only ensures appropriate air movement in the poultry house in provision of sufficient air movement for convective heat loss in hot conditions but also aids in maintenance of appropriate air quality in terms of oxygen, carbon dioxide, ammonia and humidity. In free-range production system poultry producers should avail shades by planting shrubs around the homestead or building a shade. This creates a conducive environment for the scavenging chicken to shelter during the hot periods especially in the tropics. Grass cover around the poultry shed is a way to reduce the heat load on birds, similar is the case with plantation of trees around the shade [15]. Furthermore, a shiny surface on the roof can reduce heating of the house from solar radiation. Provision of appropriate shelter, shade, sprinkling systems and ventilation are the various management strategies employed. To avoid compounding the effect of heat stress in poultry flocks, additional stress which could result from routine handling procedures such as vaccination, beak trimming, and transfers. If it is necessary, they should be effected during the cool hours of the day and if possible at night while making sure the birds are held gently and calmly.

Findings presented in a study by Bhadauria [14] indicates that heat stress can be reduced or eradicated by ensuring that birds are provided with the recommended floor space which ensures that there is no overstocking. A floor space of 0.06 m^2/bird for birds weighing 1.7 kg, 0.13 m^2/bird for those weighing 3.5 kg both of which translate to a density of 27.8 kg/m^2.

Besides the direct negative impacts of heat stress in poultry flocks, it has also been noted that hatchery eggs should be transported and stored in air-conditioned environment so as to maintain their quality [55]. It is noteworthy that good quality eggs have a high hatchability. Consumer eggs also need to be transported in an environment not detrimental to their quality as this will hamper their market price and preference [56]. Chicken meat is highly perishable under high temperatures and for that reason should maintained in a cold chain from slaughtering to the market to avoid loss of quality [57].

Animal breeding (selection) for chicken lines that perform better in environments characterised by high ambient temperatures has been identified as a potential heat stress management strategy [11, 52, 53]. This can be achieved by considering genes identified as having potential to be involved in thermal regulation. For example, the naked neck gene and the frizzle gene have been suggested in several literature potential genes to develop breeds that can cope with high ambient temperatures [11]. Other studies have also found that poultry breeds with superior thermotolerance can be developed by using thermo-tolerant genes such as frizzle gene, naked neck genes, dwarf gene and polymorphisms in heat shock proteins (HSP) genes using marker assisted selective breeding [52, 53]. The broiler lines have a high metabolic rate and are more susceptible to heat stress.

6.2 Dietary management

High ambient temperatures negatively impact nutrients intake and utilisation in chicken through different pathways [19, 27]. However, irrespective of the pathway, the net effect is poor performance in terms of production, reproduction and health. For example, results from the study carried out by Saeed et al. [15] indicated that the gut microorganisms' population and diversity was negatively affected by heat stress. The study findings indicated that the problem can be counteracted by supplementing the chicken with probiotics which have potential to restore the population and diversity in the jejunum and caecum thus resulting to microbial balance while maintaining the natural stability. This implies that prebiotics and probiotics can help in reducing heat stress and improving performance in poultry birds.

The study by Sohail et al. [19] showed that chronic heat stress reduced broiler production performance by interfering with the intestinal microarchitecture as well as increasing the adrenal hormone concentrations, however, this challenge was counteracted by use of prebiotics like the mannan-oligosaccharides and probiotic mixture which reduced/eradicated the negative impacts caused by the anatomical and physiological changes. An analysis of the existing literature such as [19, 25, 49, 58–61] on potential approaches has shown that nutritional manipulation as well as inclusion of feed additives such as vitamins, antioxidants, probiotics, prebiotics has potential to eradicate and/or reduce the negative impact of heat stress on chicken performance. For instance, findings presented in a study by Ranjan et al. [25] showed that varying energy concentration in chicken diets, adjusting feeding times, manipulating protein to energy ratio in chicken feeds, wet feeding and using automated drinkers raised at an optimal height positively impacted on chicken performance in environment characterised by high temperatures.

The investigation by Ranjan et al. [25] concluded that birds should be supplied with high density diets to compensate for the lost feeding time and the decreased feed intake in response to high environmental temperature. The diets should be high in amino acids, vitamins, electrolytes and sodium bicarbonate to compensate for mineral lost through increased panting. Ratriyanto et al. [61] reported that during heat stress supplementing laying quails with feed additives such as the Betane at a rate of 0.06–0.12% increased feed intake, protein and energy ratio as well as improving the egg quality variables. It is noteworthy that addition of vitamins A, E and zinc is beneficial in increasing antioxidant levels as oxidative balance is disturbed in heat stressed birds [49]. The study indicated that supplementing chicken diets with vitamin E alleviated many negative effects that occur during heat stress. Crozier et al. [58] demonstrated that use of phytochemicals with antioxidant activity helps to solve heat stress in chickens. Phytochemicals like polyphenols, a vital secondary metabolite found in certain plants serve as a means to reduce heat stress. Furthermore, it has been found that polyphenols have the ability to boost the expression of heat shock proteins (HSP) and antioxidant enzymes which restrain reactive oxygen species in the body of poultry birds [62]. Inclusion of vitamins C and E, carotenoids and microelements such as zinc, copper and selenium in chicken diet act as antioxidant non-enzyme system which helps in during stressful conditions [62]. Study by Yosi et al. [60] showed that supplementation of minerals like potassium chloride (KCl) in poultry drinking water are beneficial effect under heat stress condition.

Further, findings in the research by Sahin et al. [59] demonstrated that feeding layers late in the evening in environments constrained by high ambient temperatures resulted in improved laying percentage and egg shell quality. This would be explained by the increased nutrients intake that would support improved laying and high-quality egg shell as calcium is an integral component of chicken feeds. Literature search and review indicates that early morning or late evening chicken feeding, when the ambient temperatures are low, is a potential strategy in managing heat stress in poultry production. Wet feeding is also used in managing heat stress as it results in compensation of the water lost when birds pants, which always increase with increase in ambient temperature [19, 27]. Besides, drinking water should be placed in the shades to supply the body requirements [27]. Since water plays a key role in regulating the body temperature of birds water tanks should be located in a shade and insulated [19]. The personnel working within the poultry houses should ensure at all times that there is sufficient water flowing in the drinkers. The findings presented in Gous and Morris [27] and Sohail et al. [19] indicate that birds water intake increases by 1.2% for every 1°C rise in the temperature range of 22–32°C and 5% for 1°C rise in the temperature range of 32–38°C rises which are able to control body temperature in hot environments. Optimal utilisation of increased nutrients and water intake has to be accompanied with a good supply of oxygen [14]. This implies that there is need to enhance air flow in the chicken houses. In free range systems where ambient temperatures are high it would be advisable to place the supplemental feed near or under the shades [19].

7. Conclusion

Heat stress is one of the challenges faced by the poultry industry in the tropics and the world at large. In the recent years, global temperatures have been rising due to global warming. There are negative effects of heat stress that reduce the production and quality of poultry products. In addition, it impedes on the gains made in poultry welfare. Much information has been published concerning heat stress; nevertheless, there is need for more research on its effect and mitigation measures on free range chicken in the tropics.

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Due to the wide acceptance of poultry meat and eggs, poultry farming is the fastest growing global livestock industry. Nutrition plays a vital role in economic production and the maintenance of proper poultry health. Therefore, there is a great need to update balanced nutrient requirements for new breeds, utilize alternative feed resources, evaluate newer feed additives to optimize production while excluding antimicrobial feed additives and maintain overall health. The first section of this book contains six chapters that discuss the utilization of unconventional feeds, nanominerals to reduce mineral proportions in diets, and water intake affected by environmental temperature. The second section contains six chapters that describe proper nutritional management to improve gut health and immunity, the prevention of common diseases, and the amelioration of heat stress in poultry.

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