The crucial role of short-chain fatty acids and how phytomolecules influence them

*by **Dr. Inge Heinzl**, Editor EW Nutrition*

For optimum health, the content of short-chain fatty acids (SCFAs) is decisive. On the one hand, they act locally in the gut, on the other hand, they are absorbed via the intestinal mucosa into the organism and can affect the whole body. Newer studies in humans show a connection between the deficiency of SCFAs and the occurrence of chronic diseases such as diabetes type 2 or chronic inflammatory gut diseases.

SCFAs – what are they, and where do they come from?

SCFAs consist of a chain of one to six carbon atoms. They are crucial metabolites primarily generated through the bacterial fermentation of dietary fiber (DF) in the hindgut. However, SCFAs and branched SCFAs can also arise during protein fermentation. Short-chain fatty acids predominantly include acetate, propionate, and butyrate, which together account for over 95% of the total SCFAs, typically in a 60:20:20 ratio.

Acetate is produced in two different ways, via the acetyl-CoA and the Wood-Ljungdahl pathways where Bacteroides spp., Bifidobacterium spp., Ruminococcus spp., Blautia hydrogenotrophica, Clostridium spp. are involved. Additionally, acetogenic bacteria can synthesize acetate from carbon dioxide and formate through the Wood-Ljungdahl pathway (Ragsdale and Pierce, 2021). Acetate counts for more than 50% of the total SCFAs in the colon and is the most abundant one.

Propionate can also be produced in two ways. If it is produced via the succinate pathway involving the decarboxylation of methyl malonyl-CoA, the essential bacteria are Firmicutes and Bacteroides. In the acrylate pathway, lactate is converted to propionate. Here, only some bacteria, such as Veillonellaceae or Lachnospiraceae, participate.

Butyrate is produced from acetyl-CoA via the classical pathway by several Firmicutes. However, also other gut microbiota such as Actinobacteria, Proteobacteria, and Thermotogae, which contain essential enzymes (e.g., butyryl coenzyme A dehydrogenase, butyryl-CoA transferase, and butyrate kinase) can be involved. Butyrate can also be produced via the lysine pathway from proteins.

Besides the production of SCFAs from dietary fiber, there is another possibility for the synthesis of SCFAs as well as branched SCFAs – the fermentation of protein in the hindgut. This is something we want to avoid, since it´s clear signal of incorrect animal nutrition. It tells us that there is either oversupply of protein or decrease in protein digestion and absorption.

Which roles do SCFAs play?

SCFAs play a crucial role in the maintenance of gut health. Some benefits originate from these substances' general character, while others are specific to one acid. If we talk about the benefits of all SCFAs, we can mention the following:

1. Primarily, SCFAs are absorbed by the intestine and serve enterocytes as an essential substrate for energy production.
2. By lowering the pH in the intestine, SCFAs inhibit the invasion and colonization of pathogens.
3. SCFAs can cross bacterial membranes in their undissociated form. Inside the bacterial cell, they dissociate, resulting in a higher anion concentration and bactericidal effect (Van der Wielen et al., 2000)
4. SCFAs repair the intestinal mucosa
5. They mitigate intestinal inflammation by G protein-coupled receptors (GPRs).
6. They enhance immune response by producing cytokines such as IL-2, IL-6, IL-10, and TNF-α in the immune cells. Furthermore, they enhance the differentiation of T-cells into T regulatory cells (Tregs) and bind to receptors (Toll-like receptor, G protein-coupled receptors) on immune cells (Liu et al., 2021).
7. SCFAs are involved in the modulation of some processes in the gastrointestinal tract, such as electrolyte and water absorption (Vinolo et al., 2011)

After seeing the general characteristics of short-chain fatty acids, let us take a closer look at the specialties of the single SCFAs.

Acetate might play a crucial role in the competitive process between enteropathogens and bifidobacteria and help to build a balanced gut microbial environment (Liu et al., 2021). Additionally, acetate promotes lipogenesis in adipocytes (Liu et al., 2022).

Concerning general health, acetate inhibits, e.g., lung inflammatory response and the reduced air-blood permeability induced by avian pathogenic E. coli-caused chicken colibacillosis (Peng et al., 2021).

Propionate is thought to be involved in controlling intestinal inflammation by regulating the immune cells assisting and, consequently, in maintaining the gut barrier. Furthermore, propionate regulates appetite, controls blood glucose, and inhibits fat deposition in broiler chickens (Li et al., 2021).

In a trial conducted by Elsherif et al. (2022), birds fed a diet with 1.5 g sodium propionate/kg showed considerably ($P<0.05$) longer and wider guts, higher counts of lactobacillus($P<0.05$) and no colonization of Clostridium perfringens. The immunological state improved significantly ($P<0.05$), which could be seen by the higher antibody titers when the birds were vaccinated against Newcastle disease or avian influenza.

Butyrate additionally improves the function of the intestinal barrier by regulating the assembly of tight junctions (Peng et al., 2009) and stimulating cell renewal and differentiation of the enterocytes. Butyrate-producing microbes on their side prevent the dysbiotic expansion of potentially pathogenic E. coli and Salmonella (Byndloss et al., 2017; Cevallos et al., 2021) by stimulating PPAR-γ signaling. This leads to the suppression of iNOS synthesis and a significant reduction of iNOS and nitrate in the colonic lumen. Furthermore, the microbiota-induced PPAR-γ-signaling inhibits dysbiotic Enterobacteriaceae expansion by limiting the bioavailability of oxygen and, therefore, respiratory electron acceptors to Enterobacteriaceae in the colon.

In a trial conducted by Xiao et al. (2023), sodium butyrate enhanced broiler breeders' reproductive performance and egg quality due to the regulation of the maternal intestinal barrier and gut microbiota. Additionally, it improved the antioxidant capacity and immune function of the breeder hens and their offspring.

SCFAs' production can be managed

The extent of production depends on the diet and the composition of the intestinal flora. Nutritional strategies can be taken to regulate the production of short-chain fatty acids by providing dietary fiber and prebiotics, the respective bacteria but also additives in the diet or, on the other, negative way, use of antibiotics.

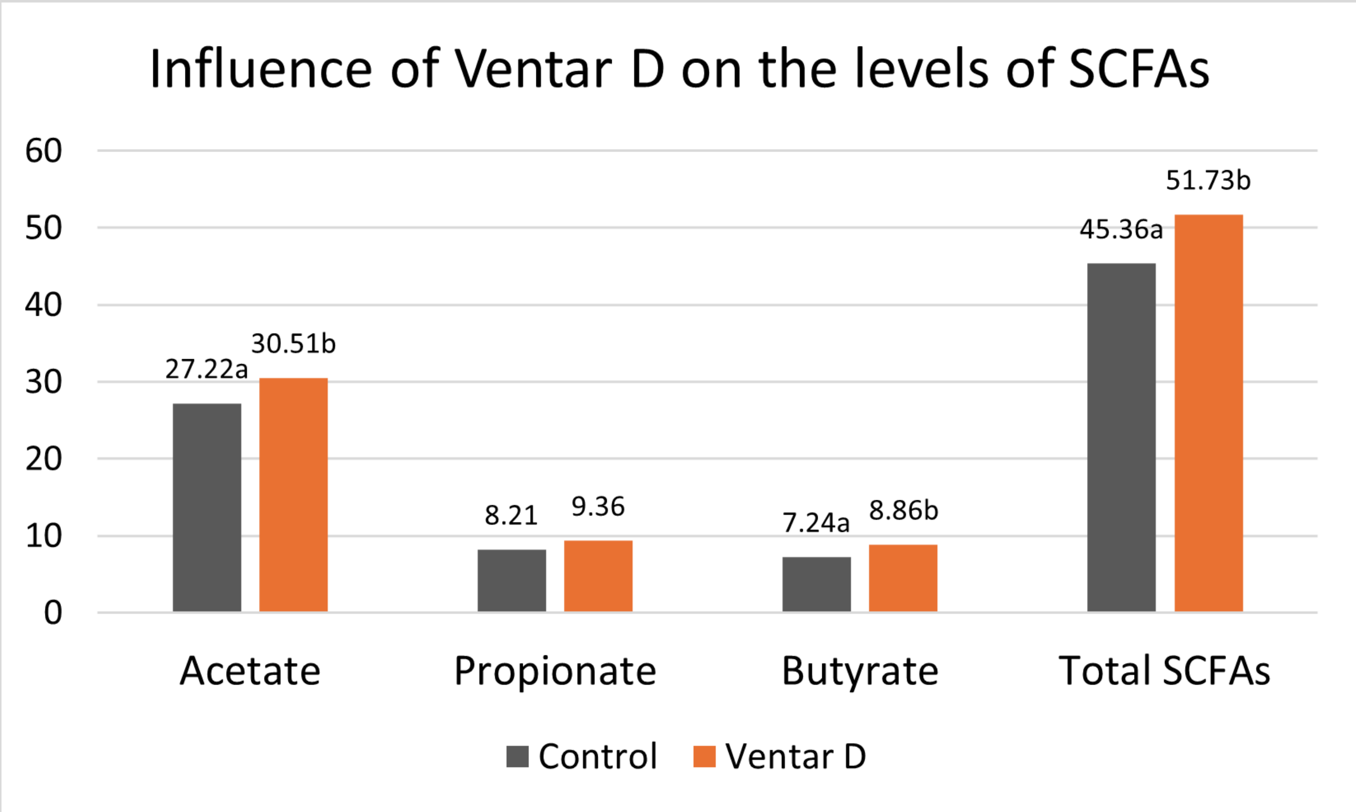
One example of SCFA-promoting additives is phytomolecules. Ventar D, a blend of diverse gut health-promoting phytomolecules, shows its SCFAs-increasing effect in a trial with Ross 308 broilers.

Trial design: The 41-day research study was conducted at an R&D farm in Turkey, with 3200 Ross 308 broilers in total. The day-old broiler chicks were randomly divided into two groups with 8 replicates in 16-floor pens (6.5×2 m each), each of 200 chicks (100 males and 100 females). One group was managed as a control group with regular feed formulation, and the other group was supplemented with Ventar D. All the birds were provided feeds and water ad libitum. Temperature, lighting, and ventilation were managed as per Ross 308 recommendation.

Groups	**Application dose**	
	Starter (crumbles)	**Grower & Finisher – 1 & 2 (pellet)**
Control	No additive	
Ventar D	100 gm/MT	100 gm/MT

All the birds and feed were weighed on days 0, 11, 23, and 41. Dead birds were also weighed, and the feed consumption was corrected accordingly. At the end of the experiment, one male and one female chicken close to the average weight of each pen were separated, weighed, and slaughtered. Short-chain fatty acid (SCFA) concentration in the caecum was measured by gas chromatography (Zhang et al. 2003). Statistical analysis of the data obtained in this study was carried out in the Minitab 18 program using the T-test following the randomized block trial design ($P \leq 0.05$). The research results were subjected to statistical analysis on a pen basis. Mortality results were evaluated with the Chi-square test.

Results: Ventar D significantly increased the levels of acetate, butyrate, and total SCFAs. The level of propionate was numerically higher. Additionally, higher final body weights (on average 160 g), improved feed efficiency (6 points), a higher EPEF (33 points), and lower mortality (0.5%) could be asserted in this experiment.



One explanation could be the microbiota-balancing effect of Ventar D. Meimandipour et al. (2010), for example, saw in their study that increased colonization of Lactobacillus salivarius and Lactobacillus agilis in cecum significantly increased propionate and butyrate formation in caeca.

Phytomolecules: Balancing intestinal microbiome and increasing healthy SCFAs

By promoting beneficial intestinal bacteria and fighting the harmful ones, phytomolecules drive the microbiome in the right direction and promote the production of short-chain fatty acids. Their gut health-protecting effect, in turn, provides for adequate digestion and absorption of nutrients, leading to optimal feed conversion and growth rates. The support of the immune system and the promotion of the antioxidant capacity additionally enhance the health of the animals. Healthy animals grow better, which ultimately leads to a higher profit for the farm.

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Mycotoxins in poultry – External signs can give a hint

Part 4: Paleness

By Dr. Inge Heinzl, Editor and Technical Team, EW Nutrition

We already showed bad feathering, mouth and beak lesions, bone issues, and foot pad lesions as signs of mycotoxin contamination in the feed, but there is another indicator: paleness. Paleness can signify a low count of red blood cells resulting from blood loss or inadequate production of these cells. Other possibilities are higher bilirubin levels in the blood due to an impaired liver, leading to jaundice or missing pigmentation.

Hen with pale comb and wattles (adapted from Bozzo et al., 2023)

The mycotoxins mainly causing anemia are Aflatoxins, Ochratoxin, DON, and T-2 toxin

Anemia can be diagnosed using parameters such as red blood cell count, hemoglobin levels, and hematocrit/packed cell volume (PCV). Numerous studies have examined the impact of mycotoxins on hematological parameters. They reveal their propensity to affect red blood cell production by impairing the function of the spleen and inducing hematological alterations. On the other hand, anemia can be caused by blood loss. Due to affecting coagulation factors, mycotoxins can lead to internal hemorrhages. The gut wall damage, probably due to secondary infections such as coccidiosis and necrotic enteritis, can entail bloody diarrhea in various animal species.

Impact on the production of blood cells

Low values of blood parameters such as red blood cells, hemoglobin, and hematocrit can result from inadequate production due to impacted production organs. The World Health Organization (WHO, 1990) and European Commission (European Commission, 2001) have identified hematopoietic tissues as targets for necrosis caused by T-2 toxin. Chu (2003) even stated that "the major lesion of T-2 toxin is its devastating effect on the hematopoietic system in many mammals, including humans". Pande et al. (2006) suggested that reduced hemoglobin values result from decreased protein synthesis due to mycotoxin contamination, a notion supported by Pronk et al. (2002), who described trichothecenes as potent inhibitors of protein, DNA, and RNA synthesis, particularly affecting tissues with high cell division rates. Additionally, the European Commission (2001) highlighted the sensitivity of red blood cell progenitor cells (in this trial, the cells of mice, rats, and humans) to the toxic effects of T-2 and HT-toxins. DAS also seems to attack the hematopoietic system, as shown in humans (WHO, 1990). A further cause for anemia might be low feed intake or nutrient absorption, which inhibits adequate iron absorption and leads to iron deficiency. In their case report, Bozzo et al. (2023) assumed that renal failure and a resulting impaired excretion capacity caused by OTA might even increase the half-life of the toxins. This would enhance their effects on their target organs, such as the liver and bone marrow, and lead to anemia.

Several studies utilizing different animal species and mycotoxin dosages have been conducted to assess the effects of Aflatoxins, Ochratoxin, and T-2 Toxin on hematological parameters. The following table provides a summary of some of these studies.

Animal species	Dosage	Impact	Reference
T-2 Toxin and other Trichothecenes			
Broilers	T-2 – 0, 1, 2, and 4 mg T-2 toxin/kg n=30 per group	Significant reduction in hemoglobin at 1, 2, and 4 ppm; PCV significantly reduced at 4 ppm	Pande et al., 2006
Broilers	T-2 – 0 and 4 mg/kg diet n=60 per group	Decrease in hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration	Kubena et al., 1989a
Broilers	4, 16, 50, 100, 300 ppm for seven days n=5-20 chickens per group	Anemia; significant reduction of hematocrit (50 and 100 ppm); survivors had atrophied lymphoid organs and were anemic	Hoerr et al., 1982
Yangzhou goslings	0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 mg/kg; n=6 per group	Red blood cell count decreased in the 2.0 mg/kg group along with an increase in mean corpuscular hemoglobin ($p<0.05$) and reduced mean platelet volume ($P<0.05$)	Gu et al., 2023

Broilers	2 ppm; 32 birds per group	Anemia, as indicated by significantly (P<0.05) lower total erythrocyte count (TEC) values, lower hemoglobin levels, and packed cell volume; additional thrombocytopenia could be the cause of bleeding	Yohannes et al., 2013
DON			
Broilers	5 and 15 mg/kg of feed for 42 days	Decrease in erythrocytes, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) at 15 mg/kg; decrease in hematocrit and hemoglobin at both levels of DON.	Riahi, 2021
Piglets	0.6 mg/kg and 2.0 mg/kg	Significant decrease in mean corpuscular volume	Modrá et al., 2013
Broilers	16 mg/kg diet n=60 per group	Significant decrease in mean corpuscular volume	Kubena et al., 1989c
Ochratoxin			
Broilers	2 mg/kg diet singly or combined with DAS 6 mg/kg	Reduced mean corpuscular hemoglobin values	Kubena et al., 1994
Broilers	2 mg/kg diet	Significant decrease in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration	Kubena et al., 1989b
Aflatoxins			
Broilers	2.5 µg/g	Decrease in red blood cell count	Huff et al., 1988
Broilers	≥1.25 µg/g	Significant decrease in hemoglobin and erythrocyte count	Tung et al., 1975
AFB1 + OTA			
Laying hens	Natural feed contamination OTA – 31 ± 3.08 µg/kg and AFB1 – 5.6 ± 0.33 µg/kg dry weight	Anemia signs (pale appearance of combs and wattles), evidenced by the discoloration of the content of the femoral medullary cavity.	Bozzo et al., 2023

Table 1: The effects of different mycotoxins on hematological parameters – hematopoiesis

In their meta-analysis, Andretta et al. (2012) reported that the presence of mycotoxins in broiler diets decreased the hematocrit and the hemoglobin concentration by 5% and 15%, and aflatoxin alone decreased the parameters by 6% and 20%.

It should be evident that a simultaneous occurrence of several mycotoxins even aggravates the situation. In an experiment involving Sprague Dawley rats, administering T-2, DON, NIV, ZEA, NEO, and OTB decreased hematocrit and red blood cell counts across all mycotoxins. However, for DON, NIV, ZEN, and OTB, red blood cell values showed partial recovery after 24 hours (Chattopadhyay, 2013). Perhaps the organism learns to cope with the mycotoxins.

The examples show that Trichothecenes, such as T-2 toxin, DON, and others, as well as Ochratoxins and Aflatoxins, impact blood parameters such as hematocrit, hemoglobin, red blood cell count, and mean corpuscular volume. All these changes might lead to paleness of the skin and birds' feet and combs.

Blood loss caused by bleeding or destruction of erythrocytes

The second possibility for anemia is blood loss due to injuries or lesions. In addition to directly causing hemorrhages, mycotoxins can promote secondary infections such as coccidiosis, which damages the gut and may produce bloody feces.

Parent-Massin (2004) e.g. reports on rapidly progressing coagulation problems after the ingestion of trichothecenes leading to septicemia and massive hemorrhages. Table 2 shows more examples of mycotoxins causing paleness due to blood loss.

Animal species	Dosage	Impact	Reference
T-2 Toxin and other Trichothecenes			
Cats	T-2 toxin – 0.06-0.1 mg/kg body weight/day	Bloody feces, hemorrhages	Lutsky et al., 1978
Cats	T-2 toxin – 0.08 mg/kg BW every 48 h until death	Bloody feces	Lutzky and Mor, 1981
Pigeon	DAS in oat, sifting	Emesis and bloody stools	Szathmary (1983)
Calves	0.08, 0.16, 0.32, or 0.6 mg/kg BW per day for 30 days; 1 calf per treatment	Bloody feces at doses ≥0.32 mg/kg BW per day	Pier et al., 1976
Ochratoxin			
Rats	Single dosages of 0, 17, or 22 mg/kg BW in 0.1 Mol/L $NaHCO_3$, gavage	Multifocal hemorrhages in many organs	Albassam et al., 1987
DON			
Broilers	0, 35, 70, 140, 280, 560, and 1120 mg/kg body weight	Ecchymotic hemorrhages throughout the intestinal tract, liver, and musculature; relationship to hemorrhagic anemia syndrome seems warranted	Huff et al., 1981
Sterigmatocystin (ST)			
10-12-day old chicks (93-101 g)	10 and 14 mg/kg BW intraperitoneal	Hemorrhages and foci of necrosis in the liver	Sreemannarayana et al., 1987
Aflatoxins			
Broiler chickens	100 µg/kg feed	Hemorrhages in the liver	Abdel-Sattar, 2019
Turkeys	500 and 1000 ppb in the diet	Bloody diarrhea, spleens with hemorrhages, petechial hemorrhages in the small intestine	Giambrone et al., 1984
Broilers	0, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg of diet combined with Infectious Bursal Disease	Slight hemorrhages in the skeletal muscles; decreased hematocrit and hemoglobin due to hemolytic anemia.	Chang and Hamilton, 1981
Broilers	0, 1, and 2 mg AFB1/kg of diet	Downregulation of the genes involved in blood coagulation (coagulation factor IX and X) and upregulation of anticoagulant protein C precursor, an inactivator of coagulation factors Va and VIIIa, and antithrombin-III precursor with 2 mg/kg	Yarru, 2009

Pigs	1-4 mg/kg, 4 weeks 0.4-0.8 mg/kg, 10 weeks	Hemorrhages	Henry et al., 2001

Table 2: The effects of different mycotoxins on hematological parameters – blood loss

Poor pigmentation

The fourth reason for paleness can be inadequate pigmentation. According to Hy Line (2021), the so-called pale bird syndrome is characterized by poor skin and egg yolk pigmentation and is caused by reduced absorption of fat and carotenoid pigments in compromised birds. This is also the case when the diets contain pigment supplements. Tyczkowski and Hamilton (1986) observed in their experiment with chickens exposed to doses of 1-8 µg of Aflatoxins/g of diet for three weeks that aflatoxins can cause poor pigmentation in chickens, probably by impairing carotenoids absorption but also transport and deposition. Osborne et al. (1982) asserted that carotenoids were significantly ($P<0.05$) depressed by 2 ppm ochratoxin as well as by 2.5 ppm aflatoxin in the diet.

Another possibility is oxidative stress due to the mycotoxin challenge. As pigments also serve as antioxidants, they may be expended for this purpose and are no longer available for pigmentation.

Paleness in poultry – a reason to think about mycotoxins

Paleness can have different causes, some of which are influenced by mycotoxins. If your chickens or hens are pale, checking the feed concerning mycotoxins is always recommended. A feed analysis can give information about possible contamination (see our tool MasterRisk).

In the case of contamination, effective products binding the mycotoxins and mitigating the adverse effects of these harmful substances can help protect your birds. As paleness is usually not the only effect of mycotoxins but also a decrease in growth, toxin binders can help maintain the performance of your animals.

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Unlocking Optimum Poultry

Performance: Harnessing the Power of GH10 Xylanase

Author: Ajay Bhoyar, Global Technical Manager, EW Nutrition

Exogenous feed enzymes are increasingly utilized in poultry diets to manage feed costs, mitigate the adverse effects of anti-nutritional factors, and enhance nutrient digestion and bird performance. These enzymes are primarily employed to bolster the availability of nutrients within feed ingredients. Among the various enzymes utilized, those capable of breaking down crude fiber, starch, proteins, and phytates are commonly integrated into animal production systems.

In monogastric animals such as poultry and swine, a notable deficiency exists in the endogenous synthesis of enzymes necessary for the hydrolysis of non-starch polysaccharides (NSPs) like xylan (McLoughlin et al., 2017). This deficiency often manifests in poultry production as a decline in growth performance, attributed to increased digesta viscosity arising from the prevalence of NSPs in commonly utilized poultry feed ingredients. Without sufficient endogenous enzymes to degrade xylan, NSPs can increase digesta viscosity, encase essential nutrients, and create a barrier to their effective digestion. In response to this issue, monogastric animal producers have implemented exogenous enzymes such as xylanases into the feeds for swine and poultry to degrade xylan to short-chain sugars, thus reducing intestinal viscosity and improving the digestive utilization of nutrients (Sakata et al., 1995; Aragon et al., 2018)

Understanding Xylanase Enzymes

Xylanase enzymes belong to the class of carbohydrases that specifically target complex polysaccharides, such as xylan, a backbone nonstarch polysaccharide (NSP) prevalent in plant cell walls. These enzymes catalyze the hydrolysis of xylan into smaller, more digestible fragments, such as arabino–xylo-oligosaccharides (AXOs) and xylo-oligosaccharides (XOs), thereby facilitating the breakdown of dietary fiber in poultry diets.

Mechanism of action

It is generally agreed that the beneficial effects of feed xylanase are primarily due to the reduction in viscosity. Studies have shown that supplementing xylanases to animal feeds reduces digesta viscosity and releases encapsulated nutrients, thus improving the overall feed digestibility and nutrient availability (Matthiesen et al., 2021). The reduction in digesta viscosity by adding xylanase is achieved by the partial hydrolysis of NSPs in the upper digestive tract, leading to a decrease in digesta viscosity in the small intestine (Choct & Annison, 1992).

GH10 vs. GH11 Xylanases

Well-characterized xylanases are mostly grouped into glycoside hydrolase families 10 (GH10) and 11 (GH11) based on their structural characteristics (amino acid composition), mode of xylan degradation, the similarity of catalytic domains, substrate specificities, optimal conditions, thermostability, and practical applications.

Why are GH10 xylanases more efficient in animal production?

While both GH10 and GH11 xylanases act on the xylan main chain, these two enzyme types have different folds, substrate specificities, and mechanisms of action (Biely et al., 2016). The GH10 xylanases are more beneficial in animal feed production due to their efficient mechanism of action, broader substrate specificity, and better thermostability, as discussed below.

GH10 xylanase exhibits broader substrate specificity

Generally, the GH10 xylanases exhibit broader substrate specificity and can hydrolyze various forms of xylan, including soluble and insoluble substrates. On the other hand, GH11 xylanases have a narrower substrate specificity and are primarily active on soluble xylan substrates. GH10 xylanases exhibit higher catalytic versatility and can catalyze the cleavage of the xylan backbone at the nonreducing side of substituted xylose residues, whereas GH11 enzymes require unsubstituted regions of the xylan backbone (Collins et al., 2005; Chakdar et al., 2016).

As a result, GH10 xylanases generally produce shorter xylo-oligosaccharides than members of the GH11 family (Collins et al., 2005). Moreover, as shown in Fig.1, the GH10 xylanase can rapidly and effectively break down xylan molecules.

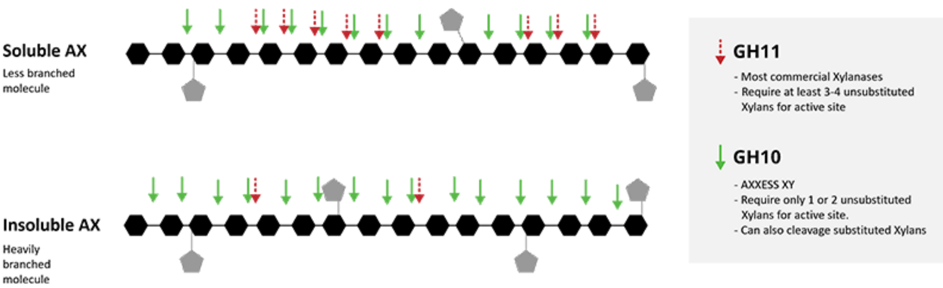

Fig.1.: Activity of a bacterial GH10 xylanase against soluble and insoluble arabinoxylans

Higher thermostability

Enzymes are proteins, and the protein's primary structure determines their thermostability. The enzyme protein tends to denature at higher than tolerable temperatures, rendering it inactive. An enzyme's high-temperature tolerance ensures its efficacy throughout the pelleted feed manufacturing. This results in consistent enzyme activity in the finished feed, subsequent gut health, and predictable performance benefits.

Xylanases with higher thermostability are more suitable for applications requiring high-temperature processes. An intrinsically heat-stable bacterial xylanase maintains its activity even under high-temperature feed processing conditions, such as pelleting.

A study conducted at the University of Novi Sad, Serbia (Fig. 2), with three pelleting temperatures (85 °C, 90 °C, and 95 °C) and conditioning times of 4 and 6 mins, showed that Axxess XY, an intrinsically thermostable GH10 xylanase, demonstrated more than 85% recovery even at 4 to 6 mins conditioning time and 95 °C temperature.

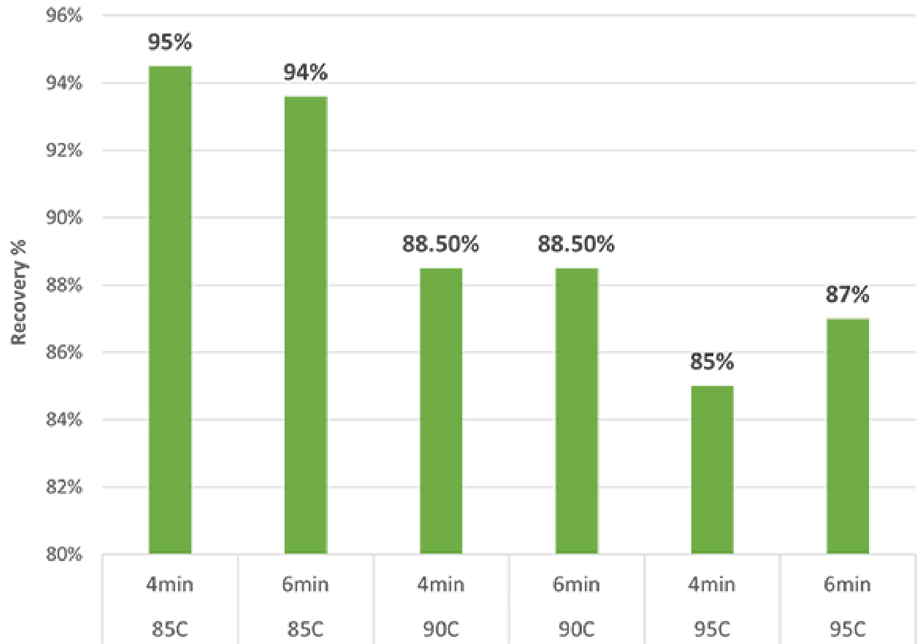

Fig.2: Optimum recovery of Axxess XY at elevated conditioning time and temperatures

Maintaining consistently optimum enzyme activity is crucial for realizing the benefits of enzyme inclusion in feed under challenging feed processing conditions.

Conclusion

In conclusion, exogenous feed enzymes, including xylanase, have gained widespread recognition for their pivotal role in poultry nutrition. The increasing use of xylanase is attributed to its ability to effectively manage feed costs while incorporating high-fiber ingredients without compromising poultry performance. However, the efficacy of xylanase is based on several factors, including its mode of action, substrate specificity, catalytic efficacy, and thermostability. Selecting the appropriate xylanase enzyme tailored for specific needs is crucial to harnessing its full benefits.

A GH10 xylanase, such as Axxess XY, designed explicitly as a feed enzyme, offers distinct advantages in poultry production. Its efficient mechanism of action, broader substrate specificity, and superior thermostability make it a preferred choice for optimizing animal performance. Notably, Axxess XY exhibits exceptional activity against soluble and insoluble arabinoxylans, thereby enhancing nutrient utilization, promoting gut health, and ultimately elevating overall performance levels in poultry.

Incorporating specialized GH10 Xylanase enzymes like Axxess XY represents a strategic approach to unlocking the nutrients in feedstuffs, ensuring optimal performance, and maximizing profitability in the poultry business.

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Low Crude Protein Diets in Poultry: Understanding the Consequences

Conference report

The concept of feeding poultry, specifically broilers and layers, with reduced crude protein (CP) diets is gaining traction among nutritionists. The economic implications of balancing amino acids currently dictate dietary CP levels. At the recent EW Nutrition Poultry Academy in Jakarta, Indonesia, Dr. Steve Leeson, Professor Emeritus at the University of Guelph, Canada, raised a crucial question: “What does ‘low CP’ really mean?” He states that it typically means a reduction of maximum 2-3% relative to current CP levels.

Low CP diets generally involve a decrease in soybean meal, compensated by higher grain content. This change increases dietary starch and decreases dietary lipid levels. To meet nutritional needs, these diets also include higher amounts of crystalline (synthetic) amino acids.

Dr. Leeson outlined the advantages and disadvantages of low CP diets. **Positives** include improved gut health due to reduced proteolytic bacteria, less environmental pollution, lower water intake (improving litter quality), improved sustainability indices, increased dietary net energy, and better performance during heat stress. **Negatives** encompass issues like lower pellet quality, altered dietary electrolyte balance, higher diet costs, reduced growth rate and feed efficiency, and increased abdominal fat deposition. There are also questions about the presumed complete utilization of crystalline amino acids, which can be as high as 25kg/MT in these diets.

Challenges with Low CP Diets

- **Protein vs. Amino Acids**: Diets are typically formulated based on digestible amino acid content, though minimum CP levels remain common, to avoid reduced performance: Dr. Leeson noted that broiler diets with less than 19% CP in starter and 15% in finisher phases, and layer diets below 13% CP, often fail to deliver adequate performance, regardless of digestibly amino acid supply.
- **Utilization of Free Amino Acids**: The crystalline amino acids are immediately absorbable in

the small intestine, contrasting with protein-bound amino acids that are absorbed as di- and tri-peptides. Amino acids absorption dynamics and endogenous loss of amino acids are affected by (high) levels of crystalline amino acids.

- **Non-Essential Amino Acids**: The impact of reduced CP on animal performance might be related to the lower levels of presumed non-essential amino acids, e.g. glycine and serine. This is an area for further exploration.
- **Energy Level Considerations**: Dr. Leeson suggests maintaining specific ratios of digestible lysine to apparent metabolizable energy in broilers at different growth stages. The heat increment of CP is an essential factor, as it reduces net energy efficiency, possibly requiring an adjustment in amino acid to metabolizable energy ratios as poultry diets are not based on net energy values.
- **Gut Health**: Lower CP levels can reduce the flow of undigested protein into the hindgut, reducing the risk of necrotic enteritis, and the production of harmful metabolites, like biogenic amines.
- **Role of Proteases**: Protease use can lead to a further 2-4% reduction in dietary CP, with the response depending on the inherent protein digestibility of the diets.
- **Impacts on Pellet Quality**: Due to the binding properties of protein, each 1% reduction in CP typically results in a 2% decrease in pellet durability (index).
- **Electrolyte Balance**: Reduced CP can significantly lower dietary electrolyte balance, which has to be considered in feed formulation. Amongst the nutrients contributing to DEB value, Sodium and Potassium appear to be the most influential minerals to consider.

Conclusion

Dr. Leeson anticipates that low CP diets will become increasingly relevant. They have the potential to reduce environmental pollution and dependence on soybean meal, despite current challenges in reducing feed costs.

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EW Nutrition’s Poultry Academy, featuring Dr. Leeson, took place in Jakarta and Manila in early September 2023. With nearly 50 years of industry experience, Dr. Leeson has made significant contributions to poultry nutrition and management, evidenced by his numerous awards and over 400 published papers.

Feed and water management strategies to mitigate heat stress in layers

Dr Daniel Valbuena, Global Manager of Technical Services, Hy-Line International – *Conference Report*

Feed and water management strategies are essential to help mitigate the negative effects of heat stress on bird welfare, production, and profitability. In EW Nutrition's Poultry Academy in September, the topic was approached in a comprehensive and practical presentation.

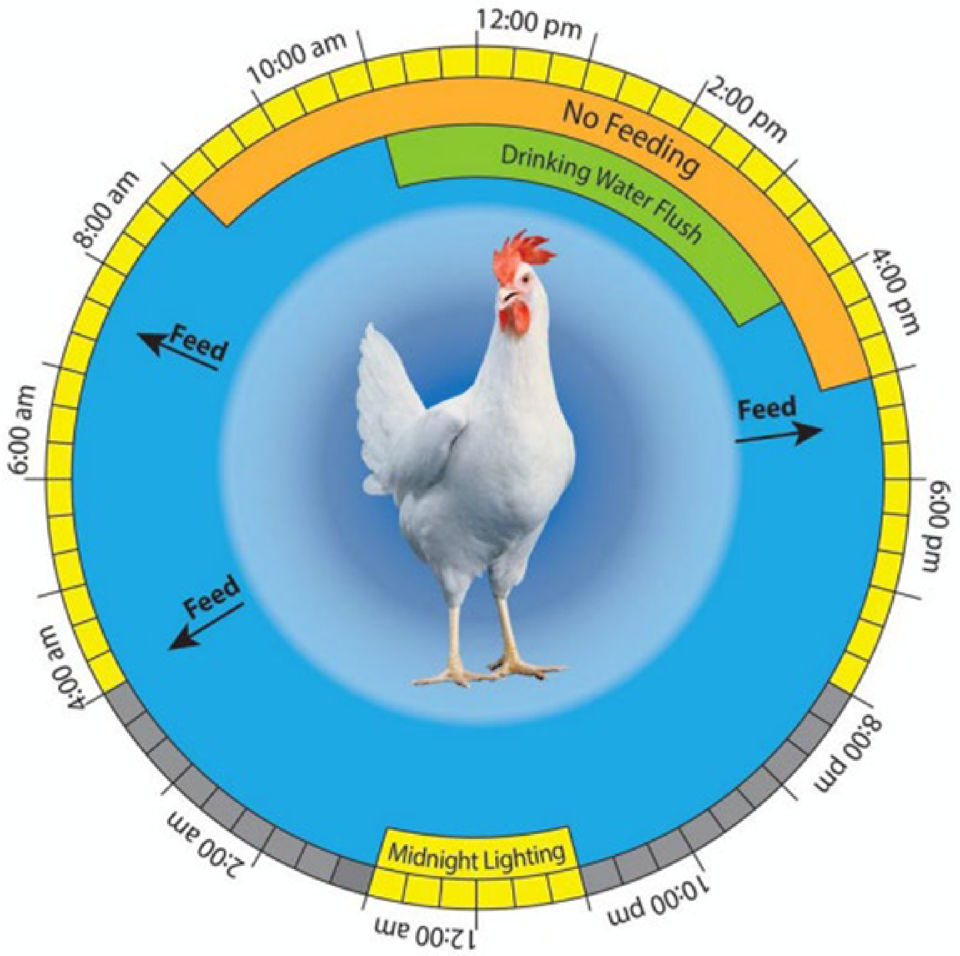
Feed management

Feed consumption of the flock should be closely monitored during hot weather. It is important to rebalance the diet for critical nutrients, particularly amino acids, calcium, sodium and phosphorous according to the birds' productivity demand (i.e., stage of production) and the observed feed intake. Insufficient amino acid intake is the primary reason for productivity loss during hot weather. Several strategies may be employed to help to manage elevated temperatures and maintain higher levels of feed intake:

- Withdrawing feed from birds 6 hours before peak hot temperatures in the afternoon can lower the risk of heat stress. Encourage as much consumption as possible in the early morning or evening. Using lighting for midnight feeding encourages feed intake.
- One third of the daily feed ration should be given in the morning and two thirds in the late afternoon. An additional advantage is the availability of calcium in the digestive system during shell formation at night and in the early hours of the morning. This will improve shell quality and reduce the birds from depleting bone calcium.
- Normally a maximum 1 hour for feeder clean-out time is recommended, but this can be extended to 3 hours when the temperature exceeds 36°C.
- Consider adding a 1-2-hour midnight feeding.
- Alter feed particle size, either by increasing it or by feeding a crumble diet. With crumble diets in laying flocks, a supplementary source or presentation of large particle limestone is recommended.
- Formulate diets using highly digestible materials, particularly protein sources. Metabolism of excess protein is particularly heat-loading on the bird. Formulate to digestible amino acid targets and do not apply a high crude protein minimum in the formula. Synthetic amino acids can reduce crude protein in the diet without limiting amino acid levels.
- Increasing the proportion of energy contribution from highly digestible lipid, rather than starches or proteins, will reduce the body heat production resulting from digestion. This is known as heat

increment and is lowest with the digestion of dietary fat.

- The bird's metabolizable energy requirement decreases as ambient temperature increases to above 21°C, resulting from a reduction of energy requirements for maintenance. The energy requirement will decrease with the rise of temperature up to 27°C, above which it will start to increase again since the bird needs additional energy for panting to reduce body heat.



Management schedule during times of heat stress

Water management

During periods of high environmental temperature, birds have a high demand for drinking water. The water-to-feed consumption ratio is normally 2:1 at 21°C but increases to 8:1 at 38°C. Adequate drinking water must be available to heat-stressed flocks. Ensure that drinkers have sufficient water flow (>70 mL/minute/nipple drinker). If water flow is less the lines need to be checked for flow restriction. If there's a build-up of iron and other minerals, it needs to be removed. Don't forget to routinely check water filters and replace them as needed.

It's easy to overlook a non-functioning drinker here and there; drinkers must be systematically checked to make sure they're all working. For floor-reared flocks, providing additional drinkers can help accommodate the increased water consumption.

During hot weather, you need to ensure your water system can accommodate the bird's increased water consumption, and the additional water demands for foggers, evaporative cooling systems and roof sprinklers. The availability of drinking water to a heat-stressed flock should never be compromised.

Cool water temperatures (<25°C) will encourage the birds to drink and reduces the birds' core temperature. Flush water lines and waterers routinely to keep the water fresh and cool, increasing water consumption, and sustaining egg production. If available, ice can also be added to header tanks. When mechanical cooling systems fail, water flushing can serve as an emergency measure during heat stress.

Drinking water from overhead water tanks can become hot if exposed to direct sunlight. These water tanks should be a light color, insulated and covered to avoid direct sunlight. Water tanks are ideally placed inside the house or underground. Water pipes in the house should not be installed close to the roof to avoid heat from the roof warming up the water in the pipes.

Having the water tank inside the house (above) or light-colored and covered to avoid direct sunlight (below) keeps the water cooler

Use vitamin (A, D, E and B complex) and electrolyte supplements in the drinking water to replenish the loss of sodium, chloride, potassium, and bicarbonate in the urine. Electrolyte supplements are best used in anticipation of a heat stress period and can be added to drinking water for up to 3 days.

Coping with evolutions in the performance and nutritional requirements of layers

***Dr. Vitor Arantes**, Global Technical Services Manager and Global Nutritionist, Hy-Line International*
– Conference Report

The layer industry has gone through significant changes during the past decades and has a remarkable capacity to cope with new challenges. Dr Vitor Arantes, Global Technical Services Manager and Global Nutritionist, Hy-Line International, noted that increased egg production, improved feed efficiency, and adaptation of egg quality and bird welfare to consumer preferences have contributed significantly to the success of the egg industry. However, continuous improvement in egg production per hen housed is the most important selection criteria in layer breeding.

Egg producers needs include:

- More saleable eggs,
- Eggshell quality,
- Easier behaviour
- Housing systems
- Egg size specifics
- Sanitary / environmental challenges
- Profits through productivity

Primary breeders can deliver these producer needs through:

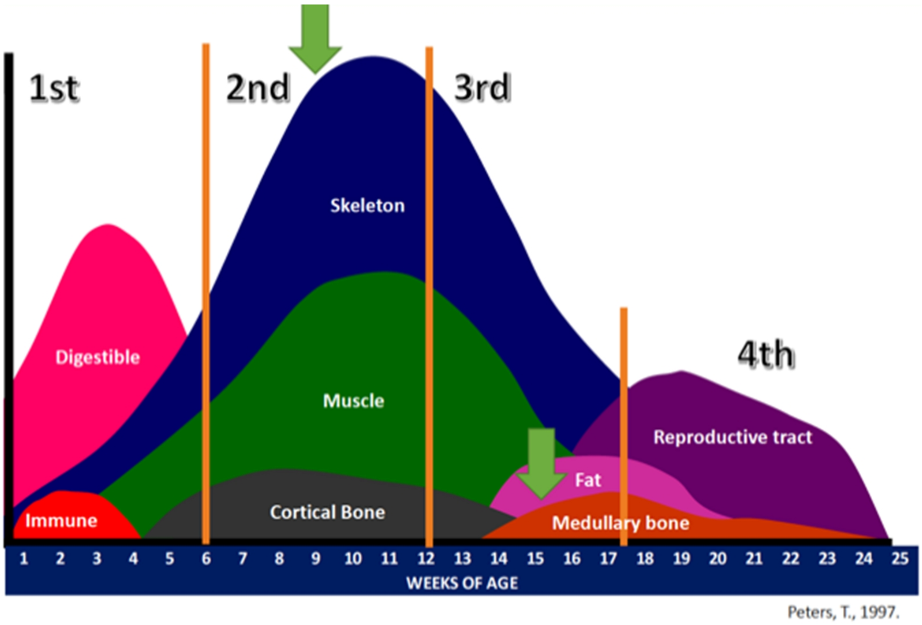
- Having the correct product for each country
- Constant follow up
- Local presence, trust relationship
- Accurate data collection
- Critical data analysis
- Understand the company's goals
- Customized technical services according to each customer needs

How has genetics changed?

Examples of genetic progress in layers from 1984 to 2022 cited by Dr Arantes include:

- Higher persistency (+30 weeks >90%)
- Higher egg mass (+5.5 kg/hen housed)
- Smaller hen (-21% mature body weight)

Dr Arantes states the record clutch size, defined as the unstopped length of individual egg production on a daily basis, was an amazing 474 days for a White Plymouth Rock hen. This genetic progress necessitates adjustments in nutrition and management.



As shown below, growth and organ development occur at various ages. "There is no margin for mistakes – a lack of growth during a stage could have a detrimental impact on pullet quality and subsequent production," stressed Dr Arantes.

Multi-phasic growth and development during rearing and start of lay

System	Age (weeks)	Consequence
Gastrointestinal	0-6	Shorter intestinal tract/reduced nutrient absorption
Immune	0-6	Flocks more susceptible to disease challenges

Skeleton	6-12	Shorter frames/less calcium reserves
Muscle	6-12	Impact in persistency of production
Fat	>12	Excess can lead to fatty liver, prone to prolapse and mortality

0-6 weeks of age

Most of the development of the organs of the digestive tract and the immune system occurs during the first 6 weeks of age. Problems that occur during this period can have negative effects on the function of these systems. Birds stressed during this period may have lifelong difficulties in digesting and absorbing feed nutrients. Immunosuppression may also result from problems during this period, leaving the bird more susceptible to diseases and less responsive to vaccinations.

6-12 weeks of age

Most of the adult structural components – muscles, bones and feathers are obtained during the period of rapid growth that occurs at 6-12 weeks of age. Growth deficiencies during this period will prevent the bird from obtaining sufficient bone and muscle reserves, which are necessary to sustain a high level of egg production and to maintain good eggshell quality. About 95% of the skeleton is developed at the end of the bird's 13 weeks of life. At this time, the plates of the long bones become calcified and further growth in bone size cannot occur.

12-18 weeks of age

During this period, the growth rate slows, and the reproductive tract matures and prepares for egg production. Muscle development continues and the proliferation of fat cells takes place. Excessive weight gain during this period can result in an excessive amount of abdominal fat. Low body weight and stressful events at this time can delay the start of egg production. From 7-10 days before oviposition of the first egg, the medullary bone that is located within the cavities of the long bones can be increased by feeding the bird a pre-laying ration with higher levels of calcium than the development stage.

Bodyweight is a key factor for flock management as this will influence future performance of birds. Consequently, bodyweight should be controlled during the whole life of the layer flocks. Management, in particular nutrition and lighting programs, can help to control bodyweight so birds can achieve their genetic potential.

Uniformity

Uniformity is the most important KPI in our business. However, with the trend towards larger flocks, maintaining uniformity is becoming more challenging. With larger flocks, it is difficult to source one unique flock which thus usually comprises multiple breeding flocks of different ages. Inevitably, uniformity will be poor, hence the need for tools to address unexpected issues. Lack of uniformity becomes a self-perpetuating cycle – dominant versus dominated.

Many egg producers use average body weights compared to the breeder recommendations as a guide to flock status. However, knowing if you have good body weight uniformity is another valuable management tool. In any flock some birds are lighter or heavier than the average body weight. Poor uniformity makes management decisions, such as lighting, feed amounts or diet phase more difficult.

Ideally, the body weight coefficient of variation (CV) should be +/-10% of the mean, increasing the likelihood that your management decision will be appropriate for most of the flock. Inappropriate diet changes, bird handling, vaccination and transfer can reduce uniformity. Flocks should be at 90% uniformity at the time of transfer to the laying facility. Body weight at point of lay significantly affected egg production and eggshell quality.

Grading into 2 or 3 sub-populations of different average bodyweights may be necessary so that each group can be managed in a way that will achieve good whole flock uniformity at the point of lay. The best predictor of future laying performance is the pullet's body weight and body type at the point of lay.

Vision egg

Vision Egg is a custom diagnostic tool used to analyze data and emphasize flock performance to achieve the highest genetic potential from Hy-Line layers with recommendations connected to customer profitability. This growing, robust database includes data from over 1 billion hens strengthens our flock performance diagnostic tool for improved profitability for Hy-Line customers.

Hy-Line customers can take advantage of this opportunity by sending flock data to their regional business manager or technical service specialist. The information shared with Hy-Line is kept completely confidential.

Summary

The challenge is not egg numbers, stated Dr. Arantes, but saleable eggs. Correct body weight and high uniformity of the flock at point of lay will result in good performance over the laying period, with high peak production and good persistency of production and the production of good quality eggs. Management is the key factor to regulation of body weight during rearing and at point of lay.

How to mitigate formulation costs when ingredient prices are high

Conference Report

The price of corn and soybeans dictates the price of all other ingredients, including to some extent amino acids, stated Dr Steve Leeson Professor Emeritus, University of Guelph, Canada at the recent EW Nutrition Poultry Academy in Jakarta, Indonesia.

The big question is, when times get tough, can we reduce safety margins and still get good performance?, asked Dr Leeson. “When we formulate diets, we build in some insurance. But so do the breeding companies in their recommendations. For sure, reducing safety margins takes us out of our comfort zones, but we need to be nutritionists, not mathematicians,” he stressed.

Protein and energy are now expensive. As a result of this economic pressure, there is a focus on strategies to reduce feed costs and improving the production efficiency and profitability of poultry enterprises. Feed cost/kg body weight gain is not always at the lowest feed:gain.

To help achieve these targets, Dr Leeson discussed feeding and management strategies that take into account the cost mitigation requirement.

Optimize current digestibility/efficiency

With high feed prices, it is especially important to review the use of feed additives that optimize nutrient release and improve ‘digestibility’. The most obvious class of such additives are the various exogenous enzymes that improve the availability of phosphorus, energy, and amino acids. In most instances, these different classes of enzymes are additive in terms of nutrient release, since they have different target substrates or modes of action. All too often, the position is taken that “I take energy uplift from my amylase, so I can’t expect energy release from phytase or protease”.

The energy release from phytase is invariably net energy related to removal of the phytate molecule, which in effect is an ‘antigen’ and takes energy to counter its negative effects. The energy release from an amylase, however, is obviously related simply to the improved digestibility of carbohydrate complexes. Similarly, a protease enzyme will always provide energy, since all protein/amino acids are eventually used for energy during protein turnover, hence our use of the often forgotten ‘n’ in AMEn. We also have the choice of enzyme concentration, especially for phytase, which in the current economic solution is likely to be close to 2 – 2.5 doses, assuming a single dose is around 500-600 FTUs. The economics of super-dosing or mega-dosing is greatly impacted by the cost of the enzyme.

The response of phytase varies with individual amino acids, and with ingredients, with greater responses with ingredients of lower inherent digestibility. Generally, Dr Leeson suggests that a protease will capture 20% of indigestible amino acids. For example:

- 70% digestibility = +6% uplift
- 90% digestibility = +2% uplift

Relax ingredient constraint maximums

Probably the greatest current cost savings can be made from relaxing the maximum levels on ingredients. While corn and soybean meal levels are usually without restriction, we often impose limits on the upper levels of ‘alternative’ ingredients such as distillers grains, rice by-products and rapeseed/canola meals, etc. When the upper levels are reached in the formula, this suggests cost savings from using higher levels. Current restraints are based on past knowledge of perhaps variable nutrient composition and so the decision to use more of any ingredient must be based on past knowledge of on-going quality control assays. Although we can achieve considerable detail today in such QC assays, monitoring for (consistency of) crude fiber, crude protein, fat, and moisture alone, provide a sound basis for decisions on whether to use more of an individual ingredient.

Source alternate ingredients

Another option is to consider ‘new’ alternative ingredients. In reality, however, there are no new ingredients as such, since all monogastric nutritionists around the world have only around 19 ingredients available in sufficient quantities to sustain large-scale modern feed mills. There are certainly smaller quantities of specialised local by-products that can be used to advantage, yet these are becoming scarce. Therefore, an ingredient is only novel to you, since inevitably the same ingredient has been used for many years in other regions. As such, there is a wealth of information available on the nutritive value of these ‘new’ ingredients that can be simply transposed to our formulation matrices.

The bird is very adaptable to new ingredients, in fact it is more responsive to nutrients. Unless there are toxins, antinutritional factors, or other negative factors, it doesn’t matter to the bird. Knowing the ingredient composition is the critical feature regarding the success or failure with new ingredients.

Reduce nutrient density

Both layers and meat birds still eat quite precisely to their energy requirements. They are amazingly adaptable to a vast range of nutrient densities, assuming that they can eat enough feed as the lower levels of feed energy are approached. Success in using lower levels of nutrient density is invariably negatively impacted by factors such as high stocking density and a high environmental temperature. Conversely, reducing diet energy usually has the hidden advantage of improved pellet quality.

The key to successful use of lower energy diets lies in prediction of change in feed intake and corresponding adjustment to all other nutrients in the diet.

Flexible cost of Dietary electrolyte balance (DEB)

When first introduced in the 1970s, maintaining DEB around 250MEq was seen to optimize broiler performance, especially leg condition. There is now less emphasis on this, perhaps because of genetic selection for skeletal integrity. DEB, however, may be important during heat stress to stimulate water intake and control manure moisture. Formulating to fixed DEB levels always adds costs. Instead, Dr Leeson suggested to focus on sodium and chloride at a ratio of 1:1.3.

Optimize feed texture (pelleting)

The first consideration is to make a good quality pellet, then worry about pellet size, noted Dr Leeson. He also added he was “a big fan of sunflower meal – it’s great for pellet quality.”

When given a choice in particle sizes, birds invariably show a preference for the largest particles. This situation becomes obvious when ‘fines’ accumulate in the feeder pans over time. As shown below, as pellet size increases, so does the bird’s need to consume fewer pellets. As a result, they need to spend less time at the feeder. Naturally, this idealised pellet size must be balanced against the willingness of mill managers to accommodate the necessary changes in pellet die size. Matching pellet size to bird age becomes critical as stocking density increases.

Impact of pellet size on pellet number consumed by a 30-day-old broiler

Pellet size (diameter)	4 mm length	6 mm length
3 mm	580	390
4 mm	330	220

5 mm	210	140

In the end, cost mitigation should not require complex mathematics. Nutritionists should be able to play with several types of improvements without affecting health and performance.

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EW Nutrition's Poultry Academy took place in Jakarta and Manila in early September 2023. Dr. Steve Leeson, an expert in Poultry Nutrition & Production with nearly 50 years' experience in the industry, was the distinguished keynote speaker.

Dr. Leeson had his Ph.D. in Poultry Nutrition in 1974 from the University of Nottingham. Over a span of 38 years, he was a Professor in the Department of Animal &Poultry Science at the University of Guelph, Canada. Since 2014, he has been Professor Emeritus at the same University. As an eminent author, he has more than 400 papers in refereed journals and 6 books on various aspects of Poultry Nutrition & Management. He also won the American Feed Manufacturer's Association Nutrition Research Award (1981), the Canadian Society of Animal Science Fellowship Award (2001), and Novus Lifetime Achievement Award in Poultry Nutrition (2011).

Meat quality is a result of genetics, feeding, the microbiome, and the handling of animals and meat

*by **Dr. Inge Heinzl**, Editor EW Nutrition*

Nowadays, nutrition is no longer about pure nutrient intake; enjoyment is also a priority. Consumers attach great importance to the high quality of food and, therefore, also of meat. The genetic selection for faster growth and feeding high-energy diets made meat production more efficient and shortened the raising period. However, this selection may sometimes also result in challenges to meat quality, such as worse water holding capacity, less marbling, less flavor, and reduced storage & processing properties.

The following article will provide detailed information about what meat quality is, how the gut microbiota influences it, and how we can increase meat quality by feeding and modulating the intestinal microflora.

Which factors can contribute to meat quality?

Meat quality is a complex term. On the one hand, meat quality covers measurable parameters such as the content of nutrients, moisture, microbial contamination, etc. On the other hand, and to no small extent, the consumers' preferences are significant. Since meat today is often sold as cuts or in parts (e.g., broiler drumsticks, breast), processing also affects the quality of meat and meat products.

Physical characteristics are objective determinants of meat quality

Physical characteristics are parameters that can be measured. For meat, the following measurable parameters determine meat quality:

1. Fat content and fatty acid composition influence tenderness and taste

Some years ago, the majority of consumers asked for completely lean meat, which, fortunately, has now changed. Fat is a flavor carrier. Especially intramuscular fat (marbling) melts during the preparation, making the meat tender, juicy, and taste good. Fat also transports fat-soluble vitamins.

A further criterion is the composition of the fat, the fatty acids. Geese fat, e.g., is known for its high content of oleic, linoleic, linolenic, and arachidonic acid, all of them derivates of the enzymatic denaturation of stearic acid (Okruszek, 2012).

One exception is cholesterol. Although belonging to the lipids and improving the sensory quality of meat, consumers prefer meat with low cholesterol content.

2. Protein and amino acid content influence the meat value

The content and the composition of protein are important factors in meat quality. Protein is essential for constructing and maintaining organs and muscles and for the functionality of enzymes. The human body needs 20 different amino acids for these tasks, eleven of which it can manufacture by itself. Nine amino acids, however, must be provided by food and are called essential amino acids. Meat is a highly valuable protein source, rich in protein and essential amino acids. The protein quality, therefore, includes the chemical and amino acid score, the index for essential amino acids, and the biological value.

In addition to the pure nutritional value, amino acids contribute to flavor and taste. These flavor amino acids directly influence meat's freshness and flavor and include threonine, alanine, serine, lysine, proline, hydroxyproline, glutamic acid (glutamate is important for the umami taste), aspartic acid, and arginine.

3. Vitamins and trace elements are essential nutrients

Meat is a primary source of B vitamins (B1-B9) and, together with other animal products such as eggs and milk, the only provider of Vitamin B12. Vitamin A is available in the innards, vitamin D in the liver and fat fish, and vitamin K in the flesh.

The most important mineral compounds in meat are zinc, selenium, and iron. Humans can utilize the iron from animal sources particularly well.

4. pH and speed of pH decline decide if the meat is suited for cooking

Since broiler chicken meat nowadays is usually consumed as cut-up pieces or processed products, the appearance at the meat counter or in the plastic box is essential for being sold. The color, seen as an apparent measurement of the freshness and quality of the meat, is influenced by the pH. The muscle pH post-mortem plays an essential role in meat quality. Due to the glycolytic process, the pH post-mortem is a good indication for evaluating physiological meat quality. A rapid pH decline post-mortem to 5.8-6.0 in most cases leads to pale, soft, and exudative (PSE) meat with reduced water retention (Džinić et al., 2015), whereas a high ultimate pH results in dark, firm, and dry (DFD) meat with poor storage quality (Allen et al., 1997)

5. Nobody wants meat like leather

The shear force is a measure of the tenderness of the meat. To determine the shear force, the meat undergoes the process of cooking and chilling. Afterward, standardized meat blocks, with fibers running along the length of the sample, are put into the Warner-Bratzler system. The blade used simulates teeth, and the system measures the force necessary to tear the piece of meat.

6. Microbial contamination is a no-go

The microbial contamination of the meat often occurs during the slaughter process. Let’s take a look at salmonella or campylobacter in poultry. The chickens take up salmonella with contaminated feed or water. Campylobacter is transmitted by infected wild birds, inadequately cleaned and disinfected cages, or contaminated water. The bacteria proliferate in the intestine. At slaughter, the intestine’s microorganisms can spread onto the meat intended for human consumption.

7. High water holding capacity is necessary to have tender meat

The moisture content contributes to the meat’s juiciness and tenderness and improves its quality. If the meat loses its moisture, it gets tough, and quality decreases. Additionally, drip loss reduces the nutritional value of meat and its flavor.

8. Fat oxidation makes meat rancid, and oxidative stress can cause myopathies in broiler breasts

Rancidity of meat occurs when the fat in the flesh gets oxidized. There are different signs of meat rancidity: bad odor, changed color, and a sticky, slimy texture. Poultry meat is considered more susceptible to the development of oxidative rancidity than red meat. This can be explained by its higher content of phospholipids, PUFAs, especially in the thighs. The breast meat, however, has a relatively low level of intramuscular fat (up to 2 %) and, additionally, myoglobin is a natural antioxidant.

But oxidative stress in broiler breasts – and this more and more happens due to a selection of always bigger breasts – can lead to muscle myopathies such as white stripes or wooden breasts, making the meat only usable for processed products.

Sensory meat quality addresses the human

senses

Besides physical quality, the sensory and chemical characteristics are essential to meat's economic importance. All attributes of meat that stimulate the human senses (vision, smell, taste, and touch) belong to the sensory quality. It, therefore, is more subjective and hard to determine. The most important features for the consumer include color (attractive or unattractive), texture (tenderness, juiciness, marbling, drip loss), and taste/ flavor (Thorslund et al., 2016).

The appearance is the first impression

Nowadays, meat is often sold as cuts lying in polystyrene or clear plastic trays, over-wrapped with transparent plastic films, so the appearance is paramount. The meat must show an attractive color. Muscle myopathies, such as the ones occurring in chickens, would not meet consumers' needs.

How does the flavor of meat develop?

There is a reaction between reducing sugars and amino acids when meat is cooked (Mottram, 1998). This Maillard reaction, along with the degradation of vitamins, lipid oxidation, and their interaction, is responsible for the production of the volatile flavor components forming the characteristic aroma and flavor of cooked meat (MacLeod, 1994). Werkhoff et al. (1990) consider cysteine and methionine the most significant contributors to meat flavor development. One factor deteriorating this quality characteristic is lipid peroxidation, which turns the taste to rancid.

Some sensory characteristics are related to physical ones

The parameters of sensory meat quality can be partly explained by measurable parameters. Water retention, e.g., influences the juiciness of the meat. The palatability increases with higher intramuscular fat or marbling (Stewart et al., 2021), the initial pH and the speed of decline decide if the flesh will be pale, soft, and exudative or normal, and lipid peroxidation is the leading cause of a decrease in meat quality (Pereira & Abreu, 2018).

Processing quality

For the processing quality, muscle structure, chemical ingredient interactions, and muscle post-mortem changes are decisive (Berri, 2000).

Does the microbiome influence the meat quality?

The gastrointestinal tract of monogastric animals disposes of a microbiome of primarily bacteria, mainly anaerobic Gram-positive ones (Richards et al., 2005). With its complex microbial community, the digestive tract is responsible for digesting feed and absorbing nutrients, but also for eliminating pathogens and developing immunity. Gut microbiotas play an essential role in digestion, are decisive concerning the synthesis of fatty acids, proteins, and vitamins, and, therefore, influence meat quality (Chen, 2022).

Intestinal microbiotas vary by species/breeds and age (Ma et al., 2022; Sun et al., 2018), and so does meat quality. For example, Duroc pigs with meat of high tenderness, good flavor, and excellent tastiness show different microbiota than other breeds (Xiao, 2017). Zhao et al.(2022) examined high- and low-fat Jinhua pigs, with the high-fat pigs showing more increased backfat thickness but also a higher fat content in the longissimus dorsi. They found low-fat pigs showed a higher abundance of Prevotella and Bacteroides, Ruminococcus sp. AF12-5, Faecalibacterium sp.OFO4-11AC und Oscillibacter sp. CAG:155, which are all involved in fiber fermentation and butyrate production. The high-fat animals showed a higher abundance of Firmicutes and Tenericutes, indicating that they are responsible for higher fat production of the organism in general but also a better fat disposition in the flesh. Lei et al. (2022) showed that abdominal fat was positively correlated with the occurrence of Lachnochlostridium and Christensenelleceae.

The intestinal microbiota-muscle axis enables us to improve meat quality by controlling intestinal microbiota (Lei, 2022). However, to develop strategies to enhance the quality of meat, understanding the composition of the microbiota, the functions of the key bacteria, and the interaction between the host and microbiota is of utmost importance (Chen et al., 2022).

Different factors influence the microbiome

Apart from that microbiotas are different in different breeds, they are additionally influenced by diseases, feeding (diets, medical treatments with, e.g., antibiotics), and the environment (climate, geographical position). This could be shown by different trials. The genetic influence on microbiota was impressively documented by Goodrich et al. (2014), who detected that the microbiomes of monozygotic twins differ less than the ones of dizygotic twins. Lei et al. (2022) compared the microbiota of two broiler breeds (Arbor Acres and Beijing-You, the last one with a higher abdominal fat rate) and found remarkable differences in their microbiota composition. When raising them in the same environment and with the same feed, the microbiotas became similar. Zhou et al. (2016) contrasted the cecal microbiota of five Tibetan chickens from five different geographic regions with Lohmann egg-laying hens and Daheng broiler chickens. Besides seeing a difference between the breeds, slightly distinct microbiota between the regions could also be noticed.

The intestinal microbiome can actively be changed by

- promoting the wanted microbes by feeding the appropriate nutrients (e.g., prebiotics)
- reducing the harmful ones by fighting them, for example, with organic acids or phytomolecules
- directly applying probiotics and adding, therefore, desired microbes to the microbiome.

An increase in the abundance of Lactobacillus and Succiniclasticum could be achieved in pigs by feeding them a fermented diet, and Mitsuokella and Erysipelotrichaceae proliferated by adding a probiotic containing B. subtilis and E. faecalis to the diet (Wang et al., 2022).

How to change the intestinal microbiome to improve meat quality?

Before changing the microbiome, we must know which microbes are “responsible” for which characteristics. However, the microbiotas do not act individually but as consortia. The following table shows a selection of bacteria that, besides supporting the gut and its functions, influence meat quality in some way.

Metabolites	Producing bacteria	Biological functions and effects on pigs
Short-chain fatty acids (acetate, butyrate, and propionate)	Ruminococcaceae Ruminococcus Lachnospiraceae Blautia Roseburia Lactobacillaceae Clostridium Eubacterium Faecalibacterium Bifidobacterium Bacteroides	Regulate lipid metabolism Improve meat quality
Lactate	Lactic acid bacteria Bifidobacterium	Important metabolite for cross-feeding of SCFA-producing microbiota

Bile acids (primary and secondary bile acids)	Clostridium species Eubacterium Parabacteroides Lachnospiraceae	Regulate lipid metabolism
Ammonia	Amino acid fermenting commensals Helicobacter	By-product of amino acid fermentation Inhibits short-chain fatty acid oxidation
B Vitamins and vitamin K	Bacteroides Lactobacillus	Serve as coenzymes in neurological processes (B vitamins) • Essential vitamin for proper blood clotting (vitamin K)

Table 1: Bacteria influencing meat quality (according to Vasquez et al., 2022)

Fat for meat quality is intramuscular fat

If we talk about increasing fat to improve meat quality, we talk about increasing intramuscular fat or marbling, not depot fat. The fat in meat-producing animals is mostly a combination of triglycerides from the diet and fatty acids synthesized. Fat deposition and composition in non-ruminants reflect the fatty acid composition of the diet but are also closely related to the design of the microbiome; short-chain fatty acids in monogastric, e.g., are exclusively produced by the gut microbiome (Dinh et al., 2021; Vasquez et al., 2022). Intramuscular fat is mainly made of triglycerides but also disposes of phospholipids associated with proteins, such as lipoproteins or proteolipids, influencing meat flavor. The fermentation of indigestible polysaccharides or amino acids results in short-chain or branched-chain fatty acids, respectively. Lactate, produced by lactic acid bacteria, is utilized by SCFA-producing microbiota. An imbalance in the microbiome fosters lipid deposition, as shown by Kallus and Brandt (2012), who found a higher proportion of Firmicutes to Bacteroidetes (50% higher) in obese mice than in lean ones. In a trial described by Zhou et al. (2016), tiny Tibetian chickens with a low percentage of abdominal fat were compared to two breeds (Lohmann layers and Daheng broilers) being large and with a high percentage of abdominal fat. The Tibetan chickens showed a two to four-fold higher abundance of Christensenellacea in the cecal microbiome. Christensenellas belong to the bacterial strain of firmicutes. They are linked to slimness in human nutrition, which was already proven by Goodrich et al. (2014) and is the contrary stated by Lei et al. (2022).

Another example was provided by Wen et al. (2023). They compared two broiler enterotypes distinguished by Clostridia vadinB60 and Rikenellaceae_RC9_gut and saw that the type with an abundance of Clostridia_vadinBB60 showed higher intramuscular fat content but also more subcutaneous fat tissue. The scientists also found another bacterium especially responsible for intramuscular fat: A lower plethora of Clostridia vadimBE97 resulted in a higher intramuscular fat content in breast and thigh muscles but not adipose tissues. Similar results were achieved in a trial with pigs and mice: Jinhua pigs showed a significantly higher level of intramuscular fat than Landrace pigs. When transplanting the fecal microbiota of the two breeds in mice, the mice showed similar characteristics in fat metabolism as their donors of feces (Wu et al., 2021).

According to several studies (e.g., Chen et al., 2008; Liu et al., 2019), intramuscular fat in chicken has a low heritability but may be controlled by feeding up to a certain extent. In pigs, Lo et al. (1992) and Ding et al. (2019) found a moderate to low (0.16 – 0.23) heritability for intramuscular fat, but Cabling et al. (2015) calculated a heritability of 0.79 for the marbling score.

At least, especially the composition of fatty acids can easily be changed in monogastric (Aaslyng and Meinert, 2017). Zou et al. (2017) examined the effect of Lactobacillus brevis and tea polyphenol, each alone or combining both. Lactobacillus is probably involved in turning complex carbohydrates into metabolites lactose and ethanol, but also acetic acid and SCFA. SCFAs are mainly produced by Saccharolytic and anaerobic microbiota, aiding in the degradation of carbohydrates the host cannot digest (e.g., cellulose or resistant polysaccharides into monomeric and dimeric sugars and fermenting them subsequently into short-chain fatty acids). Including fibers and various oligosaccharides was shown to increase the gut microbiome's fermentation capacity for producing short-chain fatty acids.

In a trial conducted by Jiao et al. (2020), they showed that SCFAs applied in the ileum modulate lipid metabolism and lead to higher meat quality in growing pigs. A plant polyphenol was used by Yu et al. (2021). The added resveratrol, a plant polyphenol in grapes and grape products, to the diet of Peking ducks and could significantly increase intramuscular fat.

Oxidation of lipids and proteins must be prevented

The composition of the fatty acids and occurring oxidative stress in adipose and muscle tissue influences or impacts meat quality in farm animals (Chen et al., 2022). During the last few years, the demand for healthier animal products containing higher levels of polyunsaturated fatty acids has increased. Consequently, the risk of lipoperoxidation has risen (Serra et al., 2021). Solutions are needed to counteract this deterioration of meat quality. As can be seen in table 1, ammonia produced by amino acid-fermenting commensals and Helicobacter inhibits the oxidation of SCFAs. Ma et al. (2022) changed the microbiome of sows by feeding a probiotic from mating till day 21 of lactation and achieved a decreased level of MDA, a sign of reduced oxidative stress. Similar results were achieved by He et al. (2022). In their trial, the supplementation of 200 mg yeast ß-glucan/kg of feed significantly decreased the abundance of the phylum WPS-2 as well as markedly increased catalase, superoxide dismutase (both $p<0.05$) and the total antioxidant activity ($p<0.01$) in skeletal muscle. Another approach was done by Wu et al. (2020) in broilers. They applied glucose oxidases (GOD) produced by Aspergillus niger and Penicillium amagasakiense. Both enzymes did not disturb but improved beneficial bacteria and microbiota. The GOD produced by A. niger reduced the content of malondialdehyde in the plasma.

Another alternative is antioxidant extracts from plants (Džinić, 2015). As consumers nowadays bet more on natural products, they would be good candidates. They are considered safe and, therefore, well-accepted by consumers and have beneficial effects on animal health, welfare, and production performance.

Hazrati et al. (2020) showed in a trial that the essential oils of ajwain and dill decreased the concentration of malondialdehyde (MDA) in quails' breast meat and, therefore, lipid peroxidation and reduced cooking loss. The antioxidant effects of thymol and carvacrol were shown by Luna et al. (2010). The group receiving the essential oils showed lower TBARS in the thigh samples than the control group but similar TBARS to the butylated hydroxytoluene-provided group.

Protein quality is a question of essential amino acids

Protein with a high content of essential amino acids is one of the most critical components of meat. Alfaig et al. (2014) tested probiotics and thyme essential oil in broilers. They found out that the content of EAAs in breast and thigh muscles numerically increased gradually from the control over the probiotic and a combination of a probiotic up to the thyme essential oil group. A significant ($p<0.05$) increase in all tested amino acids (arginine, cysteine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, and valine) could be observed in the samples of the breast and the thigh muscles when comparing the thyme essential oil group with the control. Zou et al. (2017) provided similar results, showing a significant increase in leucine and glutamic acid as well as a numerical increase in lysin, valine, methionine, isoleucine, phenylalanine, threonine, asparagine, alanine, glycin, serin, and proline through the addition of a combination of Lactobacillus brevis and tea polyphenols. They also determined an increase in the beneficial bacteria Lactobacillus and Bacteroides. The experimental results led them to the assumption that both additives may also improve the taste of meat by increasing some of the essential and delicate flavors produced by amino acids.

Tenderness is closely related to drip loss

The already mentioned trial conducted by Lei et al. (2022) with two different broiler breeds (Arbor Acres and Beijing-You) having different microbiota showed a negative correlation between drip loss and the abundance of Lachnochlostridium. They remodeled the Arbor Acres' microbiome by applying a bacterial suspension derived from the Beijing-You breed and decreased drip loss in their meat. He et al. (2022) changed the microbiome by adding yeast ß-glucan to the diet of finisher pigs. They achieved a reduced cooking loss (linear, p<0.05) and a lower drip loss (p<0.05), together indicating a better water-holding capacity, as well as a decreased lactate content. The addition of a multi-species probiotic to the diet of finishing pigs tended to result in lower cooking and drip loss(p<0.1) besides modulating the intestinal flora (higher lactobacilli and lower E. coli counts in the feces) (Balasubramanian et al., 2017) and the inclusion of Lactobacillus brevis and tea polyphenol individually or in a synergistic combination improved water holding capacity and decreased drip loss Zou et al. (2017).

Puvača et al. (2019) observed the lowest drip-loss values in breast meat and thigh with drumstick through feeding chickens 0.5 g or 1.0 g of hot red pepper per 100 g of feed, respectively, in the grower and finisher phase. The feeding of resveratrol reduced drip loss of Peking ducks' leg muscles. SCFA infused into the ileum enlarged the longissimus dorsi area and alleviated drip loss (Jiao et al, 2021).

The decrease and increase of the pH after slaughtering determines meat quality

The pH in the muscles of a living animal is about 7.2. With slaughtering and bleeding, the energy supply of the muscles is interrupted. The stored glycogen gets degraded to lactic acid, lowering the pH. Usually, the lowest pH value of 5.4-5.7 in meat is reached after 18 to 24 hours. Afterward, it starts to rise again.

In stressed animals, the stress hormones adrenalin and noradrenalin provoke a rushly occurring and, due to a lack of oxygen, anaerobic metabolism and the quick production of lactic acid. This too rapid decrease in pH leads to the denaturation of proteins in the muscle cells and reduced water-holding capacity. The result is PSE (pale, soft, and exudative) meat.

On the contrary, DFD meat (dark, firm, and dry) occurs if the glycogen reserves, due to challenges, are already used up, and the lactic acid production is insufficient. Especially PSE meat is closely related to breeds – some are more susceptible to stress, others less. However, some trials show that influencing pH in meat is possible to a certain extent.

He et al., 2022 added yeast ß-glucan to the diets of finishing pigs and a higher $pH_{45\ min}$ (linear and quadratic, p<0.01) and a higher redness (a*; linear, p<0.05) of the meat. Wu et al. (2020) achieved a significantly increased pH_{24h} through the addition of Glucose oxidase produced by Aspergillus niger.

Sensory characteristics are very subjective

In general, the sensory characteristics of meat are seen very individually. Some prefer lean, others fatty meat, some like meat with a characteristic taste, and others with a neutral. However, the typical meat taste of umami is partly determined by the nucleotide inosine monophosphate (IMP), which is regarded as an essential index for evaluating meat flavor and the acceptability of meat products. IMP provides about 40-fold higher umami taste than sodium glutamate (Huang et al. 2022).IMP is the organophosphate of inosin. Inosine, however, according to Kroemer and Zitvogel (2020), is produced by Bifidobacterium pseudolongum, which possibly can be controlled by feeding. Sun et al. (2018) compared Caoke and Partridge Shank chickens and divided them into free-range and cage groups. They found out that, except for acids, the amounts of flavor components were higher in the free-range than in the cage groups. The two housing systems also modified the microbiota, and Sun et al. took it as an indication that meat flavor, as well as the composition and diversity of gut microbiota, are closely associated with the housing systems. Fu et al. (2023) examined the addition of a mixture containing Pulsatilla, Gentian, and Rhizoma coptidis and a mixture with Codonopsis pilosula, Atractylodes, Poria cocos, and Licorice to the feed of Hungarian white geese. They saw that in both groups, the total amino acid levels, especially Glu, Lys, and Asp, increased, with, according to Liu et al. (2018), Glu and Asp directly affecting meat's freshness and flavor. Yu et al. (2021) achieved similar results by adding resveratrol to the diet of Peking ducks. The addition of the herbs additionally led to a higher Firmicutes/Bacteroidetes ratio and an increased level of lactobacilli (Fu et al., 2023).

How can EW Nutrition’s feed additives help to improve meat quality?

Meat quality is influenced by the microbiome. So, feed additives that stabilize the microbiome or promote certain beneficial bacterial strains are an opportunity.

Ventar D modulates the microbiome

Ventar D balances the microbiome by promoting beneficial bacteria such as lactobacilli and fighting harmful ones such as Clostridia, E. coli, and Salmonella. (Heinzl, 2022). In another trial with broilers, the addition of Ventar D to all feeds (100 g/t) showed an increase in short-chain fatty acids in the intestine:

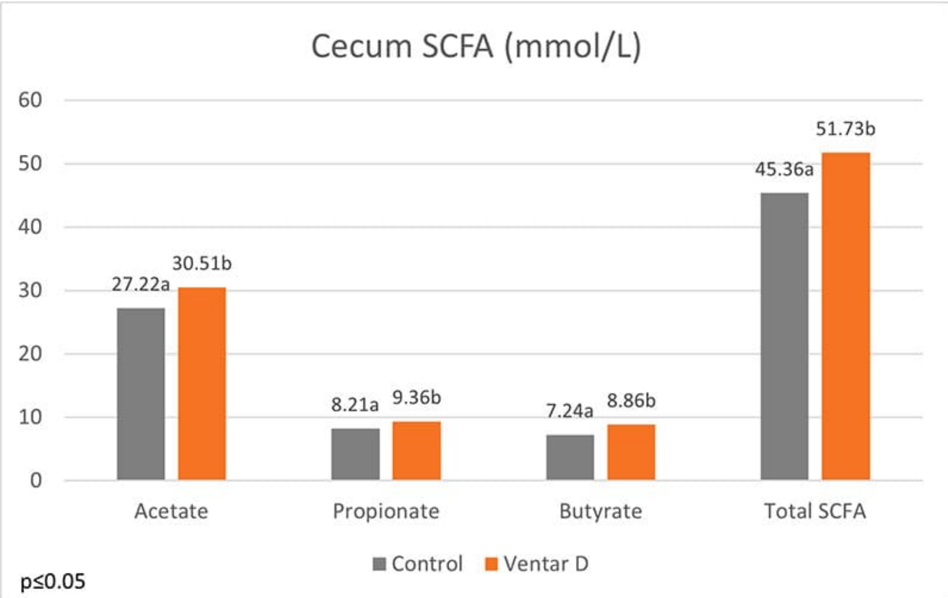


Figure 1: Short-chain fatty acids in the cecum of broilers

Santoquin countersteers oxidation

Another helpful product category is antioxidants. They can prevent the oxidation of lipids and proteins. For this purpose, EW Nutrition offers Santoquin M6*, a product tested by Kuttapan et al. (2021). Santoquin M6 was tested concerning its ability to minimize the oxidative damage caused by feeding oxidized fat. A control group receiving oxidized fat in feed was compared to one receiving oxidized fat plus 188 ppm Santoquin M6 (≙125 ppm ethoxyquin). The main parameters for this study were TBARS in the breast muscle, the incidence of wooden breast, and the live weight on day 48.

Results indicated that the inclusion of Santoquin M6 reduced the production of TBARS in the breast muscles, demonstrating a lower level of oxidative stress in the breast muscles.

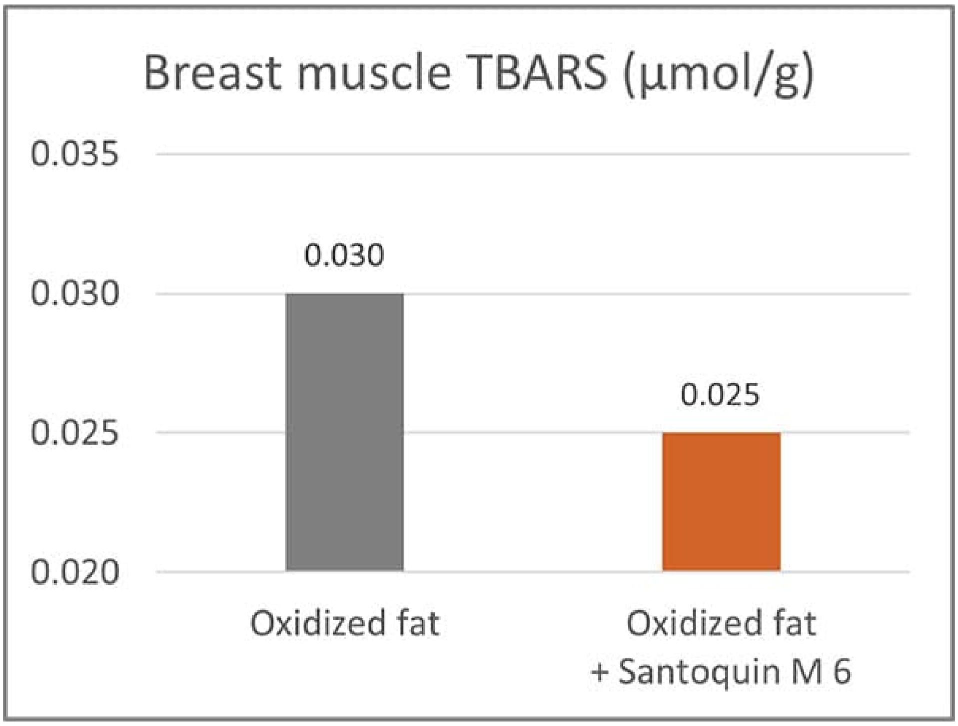


Figure 2: Thiobarbituric acid reactive substances (TBARS) in broiler breast muscles. TBARS are formed as a by-product of lipid peroxidation.

Additionally, it reduced the incidence of severe woody breasts (Score 3) by almost half and helped mitigate the impact of breast muscle degradation due to increased oxidative stress.

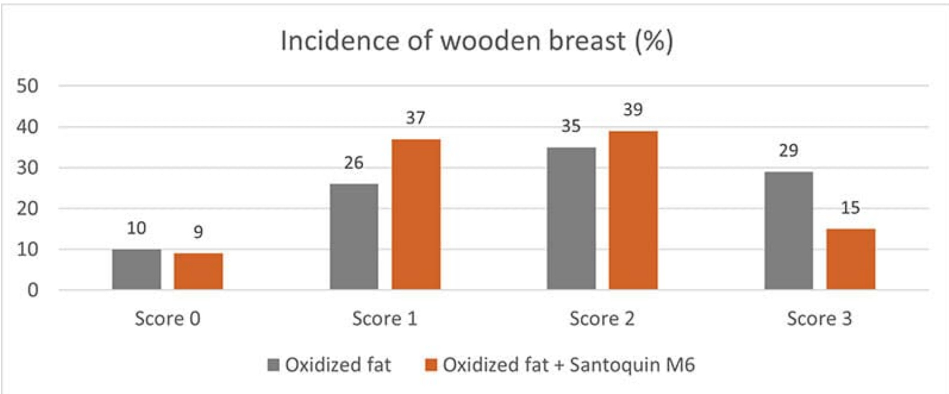


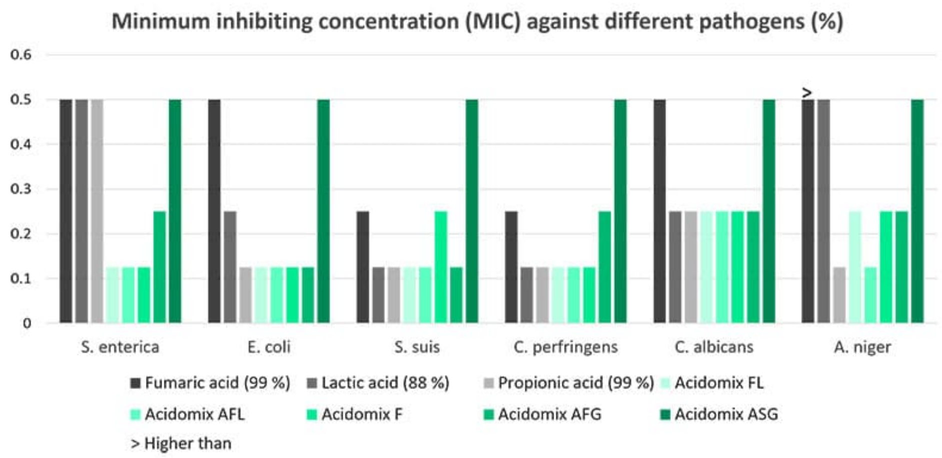
Figure 3: Incidence of wooden breast in broilers

*Usage of ethoxyquin is dependent on country regulations.

Feed hygiene with Acidomix products minimizes harmful pathogens

The Acidomix product line offers liquid, powdery, and micro-granulated products to be added to feed and water. The organic acids in Acidomix directly act against pathogens in the feed and the water and help keep the intestinal flora in balance.

A trial evaluating the effect of different Acidomix products against diverse pathogens showed lower MICs for most Acidomix products than for single organic acids. The trial was conducted with decreasing concentrations of the Acidomix products (2 – 0.015625 %) and 10^5 CFU of the respective microorganisms (microtiter plates; 50 µl bacterial solution and 50 µl diluted product).



Feeding is the one side, slaughtering the other one

With feeding, the microbiota and some meat characteristics can be changed; however, the last step, handling the animals before and the meat after slaughtering also significantly contributes to a good quality of meat. Stress due to the transport and the slaughterhouse atmosphere, combined with stress-sensible breeds, can lead to PSE meat. Incorrect handling at the slaughterhouse can lead to meat contaminated with pathogens.

Combining feeding measures with professional and calm handling of the animals is the best strategy to achieve high-quality meat.

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Feeding layers for longer laying cycles and optimized production

Conference report

At the recent EW Nutrition Poultry Academy in Jakarta Indonesia, Dr Steve Leeson, Professor Emeritus, University of Guelph, Canada, commented that "genetic progress in layer breeding has been substantial in recent decades. Since 1995, the yearly change has included +1 egg, -0.01 feed/dozen eggs, -10g final bodyweight, 0.02% mortality, and +1 week at >90% egg production. This improved persistency of commercial laying hens enables egg producers to keep flocks longer in production, provided egg shell quality can be maintained."

He noted that "the increase in hen-housed egg production is mainly due to longer clutch length and improved uniformity of layer flocks. No doubt, there is a trend in cage layers to longer production cycles. A popular commercial goal is 500 eggs in one cycle with no moult, although this has already been surpassed in many flocks. The modern layer is capable of laying 150 eggs per clutch."

Dr Leeson, however, stressed that "genetic progress and longer laying cycles have consequences. Long laying cycle programmes start during pullet rearing – you can't make decisions at 72 weeks of age. Instead, you must start with your end goals, such as persistency, egg size and shell quality, in mind. You can then develop a life-cycle approach to feeding, lighting, nutrition, and general management." Important issues to manage include:

Body weight control – early and late

Mature body weight dictates subsequent egg size. In the past, the common goal was being at, or above, management guide weight recommendations. For extended lay, a larger body weight results in too large an egg past 70 weeks of age, and so it is more difficult to maintain egg shell quality. Now the goal is to grow a slightly smaller pullet, and emphasis changes to achieving adequate early egg size from this smaller bird. This makes pre-lay nutrition for these slightly smaller pullets even more important.

The scheduling of rearing diets is more important than diet formulation. Dr Leeson's guidelines are:

- Starter diet – 19-20% CP, 2,850-2,900 kcal ME/kg from day old to target pullet body weight
- Grower diet – 17-18% CP, 2,800-2,900 kcal ME/kg from target body weight to mature body size
- Pre-lay diet (or layer diet?) – 16-18% CP, 2,800-2,900 ME/kg, mature body size to first egg

All nutrients are important, but energy is usually limiting for egg number, whereas protein/amino acids influence egg size (and feathering).

There is now even more emphasis on pullet growing to ensure adequate fat reserves through peak production, so birds are in a positive energy balance. The establishment of an energy reserve occurs during the rearing phase and has a significant effect on the bird's body composition at point of lay.

Egg size control – early and late

The obvious solution to manage body weight (and egg size) is to light-stimulate a smaller pullet, or at least to not light-stimulate a heavy pullet. This achieves a balance between accepting reduced early egg size, versus limiting an increase in egg size late in the production cycle.

Egg size can be increased in smaller early-lay pullets by:

- Reducing environmental temperature, if possible, to stimulate feed intake
- Midnight feeding 19-29 weeks
- Adequate amino acid nutrition intake, tailored to feed intake, especially methionine
- Increased number of feedings/day and increased feed particle size (pellets)

Shell strength is negatively correlated with egg size. To temper egg size late in the cycle, Dr Leeson recommended:

- Body weight control
- Controlled day length: longer day length = increased feed intake, 14 hours maximum day length in controlled-environment houses
- Warmer temperature – 26°C is ideal
- Reduce number of feedings and particle size
- Temper amino acid nutrition (with caution). Low crude protein/high amino acid diets limit the increase in egg size.

Midnight feeding provides about 1-hour extra light per day and therefore stimulating feed consumption in the middle of the dark period. Having access to feed during this period improves eggshell quality via the supply of calcium during the time when shell calcification takes place. The extra light period is perceived by the bird to be part of the night. The dark period after the light period must be longer than the initial dark period, as the bird perceives the start of the day is the end of the longest period of darkness. Removing midnight feeding should be done gradually – 15 minutes per week, advised Dr Leeson.

Preventing calcium depletion

Also known as cage layer fatigue, calcium depletion is becoming more common in all strains due to high sustained egg output. Calcium deficiency in the feed leads to loss of medullary or long bone (a reservoir of about 4g of calcium) and increased bone fragility. It is commonly seen at 35-40 weeks of age, with a 1-2% occurrence. If the incidence is more than 2%, seek advice for your pre-lay nutrition.

The development of the medullary bones takes about 10 days and requires additional calcium. Pre-lay rations support a smooth transition from developer feed to layer feed, with 2-2.5% calcium, while the other nutrients are similar to a layer feed. Pre-lay rations help the birds to adapt to the high calcium content of layer feed and to maintain sufficient daily feed intake.

To prevent calcium depletion, Dr Leeson suggested:

- Optimise pre-lay calcium (Ca) and phosphorous (P) nutrition
- Intake of 1.5g Ca, 350-450mg available P/day for at least 7 days prior to first egg

- During early lay, ensure 3.5-4 g Ca and 420 mg available P/day
- Consider vitamin D_3 water treatment (150 IU/day, twice weekly)

Pre-lay diets provide the bird with the opportunity to deposit medullary bone. This bone deposition coincides with follicular maturation and is under the control of both estrogens and androgens. The latter hormone seems essential for medullary bone growth, and its presence is manifested in the growth and reddening of the comb and wattles. Consequently, there will be little medullary deposition, regardless of diet calcium level, if the birds are not showing comb and wattle development and this stage of maturity should be the cue for increasing the bird's calcium intake.

Liver health

Excess energy relative to needs results in excess fat accumulation that is prone to oxidation. This is why you never see fatty liver haemorrhagic syndrome (FLHS) in poor-producing flocks. Layers normally have a very fatty liver, as 100% of egg yolk synthesis occurs in the liver.

The lower the fat content of the diet, the greater the stress/need to fat synthesis in the liver. With a low energy/low fat/carbohydrate diet FLHS is almost universal to varying degrees. One treatment is to add fat to the diet! Haemorrhage (not always FLHS) is inevitable with dietary omega-3s that are very prone to oxidation.

Dr Leeson recommended prevention/control for FLHS, which usually starts about weeks 36-40, including:

- +1.0 kg choline
- +0.5 kg methionine
- +100 IU vitamin E
- +30% does Hy-D because of impaired liver metabolism of vitamin D_3 (that can also impact calcium absorption)
- Add 2% dietary fat without change in diet energy level

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EW Nutrition's Poultry Academy took place in Jakarta and Manila in early September 2023. Dr. Steve Leeson, an expert in Poultry Nutrition & Production with nearly 50 years' experience in the industry, was the distinguished keynote speaker.

Dr. Leeson had his Ph.D. in Poultry Nutrition in 1974 from the University of Nottingham. Over a span of 38 years, he was a Professor in the Department of Animal &Poultry Science at the University of Guelph, Canada. Since 2014, he has been Professor Emeritus at the same University. As an eminent author, he has more than 400 papers in refereed journals and 6 books on various aspects of Poultry Nutrition & Management. He also won the American Feed Manufacturer's Association Nutrition Research Award (1981), the Canadian Society of Animal Science Fellowship Award (2001), and Novus Lifetime Achievement Award in Poultry Nutrition (2011).

Nutritional considerations for immunity and gut health

Conference report

At the recent EW Nutrition Poultry Academy in Jakarta, Indonesia, Dr Steve Leeson, Professor Emeritus, University of Guelph, Canada, opened his presentation by stating that "it is obvious that any nutrient deficiency will impact bird health, but not so obvious is that nutrition *per se* can positively impact immunity and health in an otherwise healthy and high-producing bird."

Modern high-performing broilers are characterized by extremely high feed intake. This puts a lot of stress on the physiology of the entire gastrointestinal tract, but particularly so on the absorptive epithelial cells of the small intestine. Any organism requires a nutrient source for survival and reproduction. Dr Leeson asked "can we significantly reduce nutrient supply to pathogens, while sustaining bird productivity?"

He reminded the audience that no cellular function comes for free: so there is always a "cost". A general conclusion is that 10% of nutrients can be used for immune function during disease challenge, and always get priority. Therefore, you don't want to overstimulate the immune system, which in extreme situations leads to an inflammatory response. In his presentation, Dr Leeson considered factors determining gut health and nutritional tools which are available to support gut health.

Gut microflora

Gut pathogens impact the bird and/or the consumer. *Clostridia* and *E. coli* are the major concerns regarding bird health and productivity, whereas *Salmonella* and *Campylobacter* are major pathogens important for human health.

The chick hatches with a gut virtually devoid of microbes, so early colonizers tend to predominate quite quickly. Microbial species present on the hatching tray, during delivery and during the first few days at the farm will likely dictate early gut colonization. In some instances, the chick's microflora may be established by the time it gets to the farm, so the probiotic faces more of a challenge to establish itself as the predominant species.

Antibiotic alternatives

Gut villi development matures at around 10-15 days of age. The broiler pre-starter diet therefore is a target for feed additives that positively impact gut structure and development.

- Among the **short chain fatty acids**, butyric acid is considered the prime energy source for enterocytes and it is also necessary for the correct development of the gut-associated lymphoid tissue (GALT). Butyric acid can also be added indirectly via fermentation of judicious levels of soluble fiber to encourage optimal gut villi development. Dr Leeson added that he is a big believer in butyric acid, encouraging a good gut structure at 10 days, which can be worth about 50 kcal.
- **Exogenous enzymes** should also be considered in an attempt to maximize digestion and limit the flow of nutrients to the large intestine and ceca. Protease enzymes have great potential in this regard, since they allow nutritionists to reduce dietary crude protein and hopefully reduce the supply of nitrogen that fuels proteolytic Clostridia bacteria in the large intestine and ceca.
- **Amino acids**, particularly threonine, play a critical role in the maintenance of intestinal mucosal integrity and barrier function, especially for mucin synthesis, which protects enterocytes from adherence by pathogenic bacteria, and from attack by endogenous enzymes and acids.
- **Polyunsaturated fatty acids** (PUFAs) – Omega-3s and especially DHA from fish oil help to reduce inflammatory response (overstimulation). Omega-3s are poorly converted to DHA by the chicken, so conventional sources such as flax are of limited application for immunity.
- **Blood plasma** from pigs or cattle is a complex spray-dried mixture of proteins and amino acids, many of which are immunoglobulins that "temper" the immune system, much like PUFAs.
- **Vitamins A, D, E and C** have vital roles in the normal function of the immune system and have antioxidant capacity.
- Certain **complex carbohydrates**, such as ß-glucans, influence gut health due to their fermentation, leading to the production of short-chain fatty acids, such as butyrate.
- **Antioxidants** – to firstly control oxidation of fats and fat-soluble vitamins in feed, and secondly to optimize birds' cellular oxidative capacity, to prevent cell damage, therefore maintaining healthy cellular and immune function.
- **Betaine** increases intracellular water retention, reducing "dehydration" of microvilli and increasing their volume/surface area.
- **Fiber** – moderate levels (1-2%) of soluble (fermentable) and insoluble fiber can be beneficial to early gut development by stimulating gizzard development and endogenous enzyme production.
- **Phytogenics** are becoming very common in combination with acidifiers (upper tract) and probiotics. Essential oils are becoming more mainstream the more we know about them.

Recommendations for optimizing gut health and immunity

Fast growth rate and high egg output are negatively correlated with immune response. Consequently, nutrient-dense diets are not optimal for immunity. With bacteria, it's a numbers game – but these numbers quickly multiply. The first 7 days are important, therefore probiotics must be established early. Consider the role of targeted feed additives, such as butyrate, phytogenics, antioxidants, PUFAs etc.

Also, maximize feed particle size – the limit is usually pellet quality. Mitigate nutrient transition at any diet change. Review the supply of trace minerals, as there is a trend to lower levels of organic minerals. With all the factors that weigh into production performance, any support that can be rallied through nutrition needs to be considered.

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EW Nutrition’s Poultry Academy took place in Jakarta and Manila in early September 2023. Dr. Steve Leeson, an expert in Poultry Nutrition & Production with nearly 50 years’ experience in the industry, was the distinguished keynote speaker.

Dr. Leeson had his Ph.D. in Poultry Nutrition in 1974 from the University of Nottingham. Over a span of 38 years, he was a Professor in the Department of Animal &Poultry Science at the University of Guelph, Canada. Since 2014, he has been Professor Emeritus at the same University. As an eminent author, he has more than 400 papers in refereed journals and 6 books on various aspects of Poultry Nutrition & Management. He also won the American Feed Manufacturer’s Association Nutrition Research Award (1981), the Canadian Society of Animal Science Fellowship Award (2001), and Novus Lifetime Achievement Award in Poultry Nutrition (2011).