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).

Metabolic disorders and muscle defects

Conference Report

At the recent EW Nutrition Poultry Academy in Jakarta Indonesia, Dr Steve Leeson, Professor Emeritus, University of Guelph, Canada, defined metabolic disorders as: non-infectious, occurring with adequate diets in 'normal' conditions, and mostly species-specific. Their incidence is negatively correlated to productivity. Although they often have a major genetic component, genetic selection to manage the problem is often a last resort, as there is usually a negative correlation with productivity.

Ascites

First reported in the 1970s, ascites or 'water belly' is probably the number one metabolic issue today. It is the accumulation of fluid in the abdomen, which is caused by a cascade of events related to the need to supply high levels of oxygen to the tissues. The condition was initially most prevalent in fast-growing male broilers maintained at high altitude and where there is a degree of cold stress, but nowadays the problem can occur at any altitude. In extreme situations up to 8% mortality is seen, although 1-3% mortality is currently more common. The disorder is now re-emerging with faster growth rates, as growth rate is easily the main contributing factor.

Options to limit ascites include:

- Limit growth rate
- Feed texture (mash vs. pellets)
- Never let the temperature get below 15°C for any age of bird
- Brooding ventilation – economics of air flow vs. temperature

- Minimize environmental contaminants, such as dust
- Lighting programs (4-6 hours of darkness)

Sudden death syndrome (SDS)

SDS almost always affects males birds close to market weight. It frequently afflicts 1-5% of the flock and from 21-35 days it will usually be the major cause of death. Afflicted birds appear healthy, are well fleshed and invariably have feed in their digestive tract. Death occurs within 1-2 minutes, the birds most frequently being found dead on their backs. There are few changes in gross pathology. The heart may contain blood clots, that are likely post-mortem in origin, and the ventricles are usually empty. Diagnosis is usually by exclusion of other diseases. The lungs are often oedematous, although this usually occurs when birds spend time on their backs and fluid drains to the lung region by gravity. There are no specific changes in the tissue or blood profile that can be used for diagnosis. The condition is precipitated by fast growth rate, and so conversely it can be prevented by varying degrees of nutrient restriction.

Spiking mortality syndrome (SMS)

SMS is characterized by severe unexplained hypoglycemia, and always occurs from 18-21 days of age. There are few post-mortem observations, so it is often misdiagnosed. Mortality can be 2-3%. Males are more susceptible than females, probably because they are growing faster. Birds fed all-vegetable diets may be more prone to SMS. Supplementing an all-vegetable diet with milk-powder (which is high in serine), casein or serine is recommended and results in increased blood glucose.

Skeletal integrity

This disorder is not due to increased bodyweight of broilers, as the broiler is capable of supporting weight that far exceeds its own body weight. Instead, it’s due to shifting the bird’s center of gravity forward as breast muscle yields have increased, moving the legs further apart which puts torsional pressure on the head of the femur. Not only does it cause on-farm problems, but also complications with mechanical processing.

Imbalanced nutrient supply, such as excess of chloride, or infection with bacteria, viruses, and particularly mycoplasmas are involved.

Tibial dyschondroplasia (TD)

TD is due to abnormal cartilage development. Failure of normal vascularization limits mineralization. TD is characterized by enlargement of the hock, twisted metatarsi, and slipped tendons. A low electrolyte balance (<200MEq), high chloride (>0.3%), or low Ca:P or high P:Ca can precipitate TD. Adding manganese and choline to the diet will largely eliminate it.

Perosis

Now often termed Chondrodystrophy, it has manganese or choline deficiency as the classical cause, but it can also be seen with other B-vitamin deficiencies. As with TD, it can be aggravated by some grain fumigants.

Kinky back

Also known as Spondylolisthesis, it is not really a metabolic disorder, as *Enterococcus* infection is the most common cause. Chickens with kinky back syndrome are often seen sitting on their tail, extending their feet outward or letting them fall over to one side of their body. Once the condition stops birds from being able to walk, they are unable to reach food or water on their own and are at risk of dying from starvation. There

is no treatment for kinky back.

Gizzard erosion and proventriculus

Although gizzard lesions are very common, Dr Leeson suspects their importance is overemphasized. Gizzard condition is seen in both layer and broiler chickens, but the incidence is more in broilers.

Access to grit and inclusion of at least 20% cereal particles larger than 1 mm in size in the diet will have a positive effect on the development and functioning of the gizzard and it will also reduce the frequency and severity of gizzard lesions in poultry. Ingestion of non-soluble fibers has been shown to exert strong effects on the structure and function of the gizzard. Inclusion of at least 3% coarse fibers in the feed increased the relative weight of the gizzard and reduced the pH of the gizzard contents suggesting a preventive effect of fiber.

Proventriculus appears as a very large organ and is often associated with gizzard erosion. When the proventriculus glands are affected, there is a lower secretion of hydrochloric acid and enzymes and therefore more undigested feed arrives to the intestine, where it can act as a substrate of pathogens and start digestive infections.

Breast muscle defects

Breast muscle defects are not problematic for the bird, efficiency/economics of growth, or a food safety issue. The main issue is seen at primary or secondary processing, and consumer acceptance. Due to the fast muscle growth and the enlarged muscle cells, the space between muscle fibers is reduced. This restricts the blood supply to the muscles, which can no longer reach the desired oxygen levels.

White-striping

White striping is a quality factor in chicken breast meat caused by deposits of fat in the muscle during the bird's growth and development. It is like marbling in red meat. Dr Leeson joked that it be promoted as marbled chicken – like Wagyu beef. Because hypoxia is associated with white striping, it was thought that arginine supplementation could help with vasodilation, thus supplying the muscles with better oxygen resources.

Wooden breast (WB)

WB is an emerging quality defect. Macroscopically, it is characterized by palpably hard, pale ridge-like bulges at the caudal end, along with clear viscous fluid, small hemorrhages, and white striping, that may occur separately or together. The main cause is the high growth rate and high breast meat yield. There is no nutritional or management solution.

Wooden breast is common in male broilers >2.5 kg bodyweight, and the incidence tends to increase with the size of the breast fillet. As the incidence of wooden breast increases, the incidence of white striping tends to decrease. Due to the visual defects and hard and chewy texture, consumers have a low acceptance of WB fillets, and they are usually downgraded to use for ground products.

Reducing oxidative stress and supplying more oxygen to the cells, enabling the muscle cells to grow very fast without meat loss will reduce the incidence of WB.

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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:

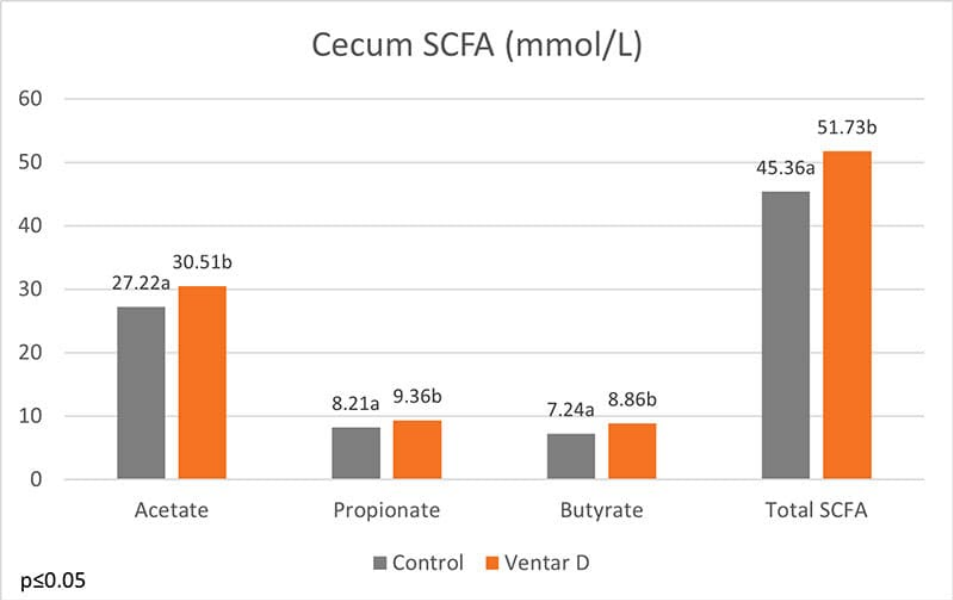


Figure 1: Short-chain fatty acids in the cecum of broilers

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.

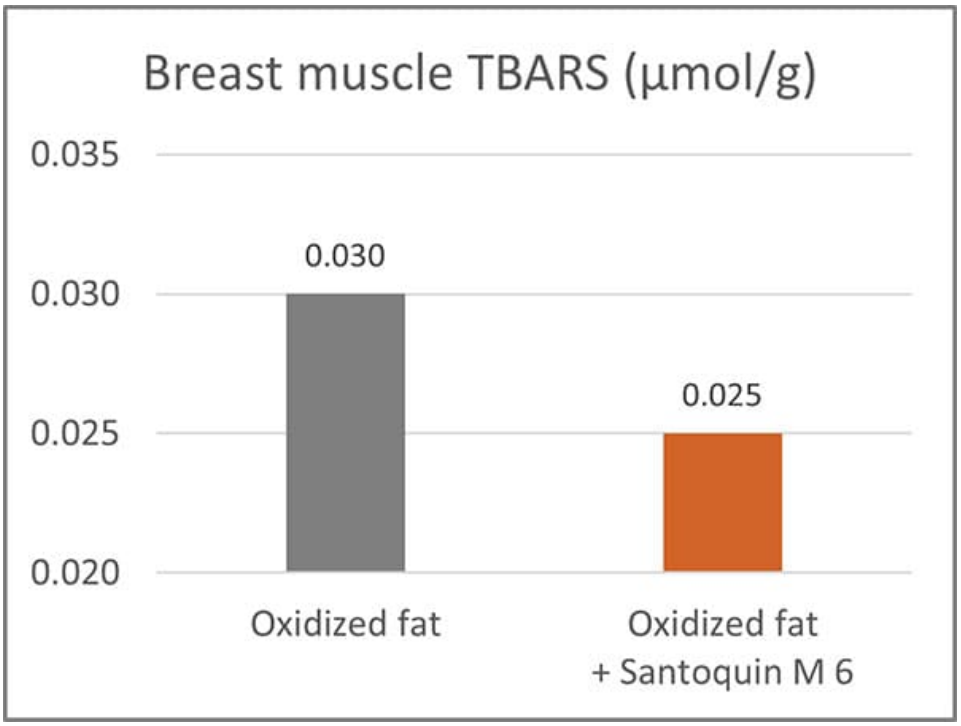


Figure 2: Thiobarbituric acid reactive substances (TBARS) in broiler breast muscles. TBARS are formed as a by-product of lipid peroxidation.

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.

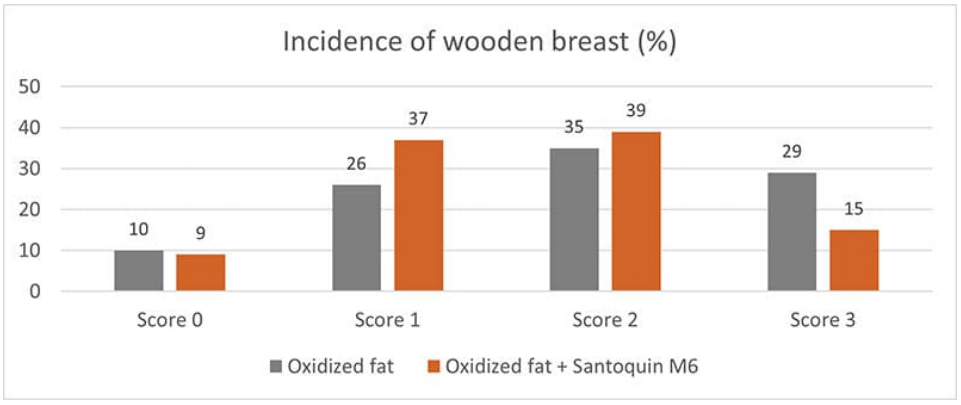


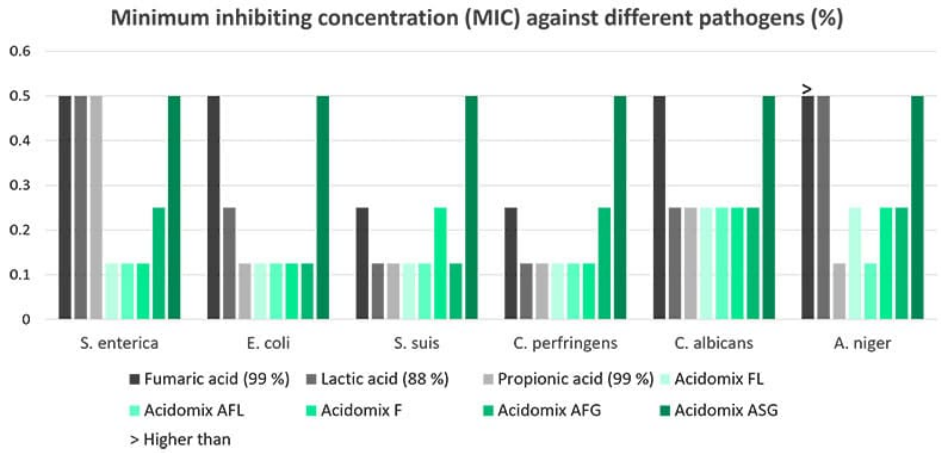
Figure 3: Incidence of wooden breast in broilers

*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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Respiratory health in poultry: no action is no solution

*by **Inge Heinzl** and **Ruturaj Patil**, EW Nutrition*

Broilers face high respiratory disease risks. In winter, they often come from lower temperatures; throughout the year, they come from improper ventilation and proximity to manure or infected birds. The confined spaces and lack of proper airflow create an environment conducive to harmful airborne particles and pathogens, significantly compromising birds' respiratory health. In the possible presence of viruses such as ILT (Infectious Laryngotracheitis Virus), IBV (Infectious Bronchitis Virus), AIV (Avian Influenza Virus, NDV (Newcastle Disease Virus), bacteria like *Mycoplasma gallisepticum*, *E. coli*, or Chlamydia, respiratory issues are inevitable.

High efficiency takes its toll

A bird, generally a flying species, has a complex respiratory system. Instead of the diaphragm cooperating with the lung, nine additional air sacs do the job of sucking in and blowing out of the air like bellows. They increase the air volume passing through the lungs, where oxygen absorption occurs. The air sacs are situated in different parts of the birds and connected to hollow (pneumatic) bones.

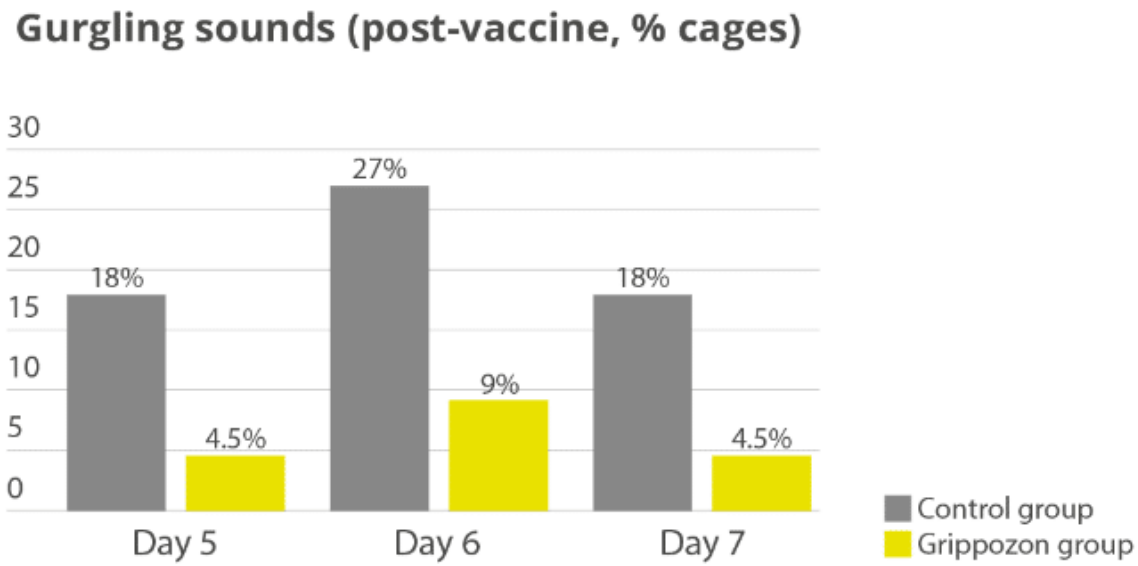
The co-action of the air sacs and the lung results in a high efficiency of the bird's respiratory system: birds can extract about 160% more oxygen from the air than mammals. However, the extended parts of the respiratory system also offer a high contact surface for pathogens. To protect themselves, the respiratory system is equipped with

– cilia in the trachea to propel entrapped particles for disposal
– mucus produced by goblet cells in the trachea and cooperating with the cilia
– immune cells in the lung, scavenging inhaled particles and bacteria that enter the lower respiratory tract

Additional support is recommended

To additionally support your birds against respiratory issues, stress should be kept low, and immunity to diseases should be high. If possible, decrease the stocking density. Effective litter management can help keep litter particle inhalation low. These particles irritate the respiratory system and reduce immune resistance. They often carry pathogens and possibly induce respiratory issues through several toxic mechanisms.

Another possibility is using phytogenic substances alone or combined with vaccines. Eucalyptus oil exerts antimicrobial, anti-inflammatory, mucolytic, and bronchodilator effects in the case of respiratory disease. Thyme has expectorant, mucolytic, antitussive, and antispasmodic characteristics, and mint, with its antihistamine and cooling effect, acts as a decongestant. Grippozon is such an example, based on fast-acting, concentrated phytomolecules supporting animals against respiratory challenges.



A trial with 20,000 birds showed fewer gurgling sounds and reduced post-vaccination reaction than the untreated group.

Regardless of the solution chosen, especially with the cold season coming and high stocking density a given in many parts of the world, by far the worst action is no action at all.

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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Decoding the connection between stress, endotoxins, and poultry health

By Technical Team, EW Nutrition

Stress can be defined as any factor causing disruptions to homeostasis, which triggers a biological response to regain equilibrium. We can distinguish four major types of stressors in the poultry industry:

- Technological: related with management events and conditions
- Nutritional: involving nutritional disbalances, feed quality and feed management
- Pathogenic: comprising health challenges.
- Environmental: changes in environment conditions

In practical poultry production, multiple stress factors occur simultaneously. Their effects are also additive, leading to chronic stress. The animals are not regaining homeostasis and continuously deviate the use of resources through inflammation and the gut barrier-function, thus leading to microbiome alteration. As a consequence, welfare, health, and productivity are compromised.

What are endotoxins?

Bacterial lipopolysaccharides (LPS), also known as endotoxins, are the main components of the outer membrane of all Gram-negative bacteria and are essential for their survival. LPS have direct contact with the bacteria's surroundings and function as a protection mechanism against the host's immunological response and chemical attacks from bile salts, lysozymes, or other antimicrobial agents.

Gram-negative bacteria are part of animals' microbiota; thus, there are always LPS in the intestine. Under optimal conditions, this does not affect the animals, because intestinal epithelial cells are not responsive to LPS when stimulated from the apical side. In stress situations, the intestinal barrier function is impaired, allowing the passage of endotoxins into the blood stream. When LPS are detected by the immune system either in the blood or in the basolateral side of the intestine, inflammation and changes in the gut epithelial structure and functionality occur.

The gut is critically affected by stress

Even when there is no direct injury to the gut, signals from the brain can modify different functions of the intestinal tract, including immunity. Stress can lead to functional disorders, as well as to inflammation and infections of the intestinal tract. Downstream signals act via the brain–gut axis, trigger the formation of reactive oxygen and nitrogen species as well as local inflammatory factors, and circulating cytokines, affecting intestinal homeostasis, microbiome, and barrier integrity.

Stress then results in cell injury, apoptosis, and compromised tight junctions. For this reason, luminal substances, including toxins and pathogens, leak into the bloodstream. Additionally, under stress, the gut microbiome shows and increment on Gram-negative bacteria (GNB). For instance, a study by Minghui Wang and collaborators (2020) found an increase of 24% in GNB and lower richness, in the cecum of pullets subjected to mild heat stress (increase in ambient temperature from 24 to 30°C).

Both these factors, barrier damage and alterations in the microbiome, facilitate the passage of endotoxins into the blood stream, which promotes systemic chronic inflammation.

What categories of stress factors trigger luminal endotoxins' passage into the bloodstream?

Technological stress

Various management practices and events can be taken as stressors by the animals' organism. One of the most common examples is **stocking density**, defined as the number of birds or the total live weight of birds in a fixed space. High levels are associated with stress and loss of performance.

A study from the Chung-Ang University in 2019 found that broilers with a stocking density of 30 birds/m^2 presented two times more blood LPS than birds kept at half of this stocking density. Moreover, the body weight of the birds in the high-density group was 200g lower than the birds of the low-density group. The study concluded that high stocking density is a factor that can disrupt the intestinal barrier.

Nutritional stress

The feed supplied to production animals is designed to contribute to express their genetic potential, though some feed components are also continuous inflammatory triggers. **Anti-nutritional factors, oxidized lipids, and mycotoxins** induce a low-grade inflammatory response.

For instance, when mycotoxins are ingested and absorbed, they trigger stress and impair immunity in animals. Their effects start in gastrointestinal tract and extend from disrupting immunity to impairing the intestinal barrier function, prompting secondary infections. Mycotoxins can increase the risk of endotoxins in several ways:

- By inducing changes in the intestinal microbiota that increase gram-negative bacteria
- By disrupting the intestinal barrier function, allowing endotoxins (as well as other toxins and pathogens) to cross the gut barrier and pass into the bloodstream
- By alterations in the immune response, low doses of mycotoxins, such as trichothecenes, induce the upregulation of pro-inflammatory cytokines. A possible synergy can be inferred as when they are together, the effects may be prolonged and require a lower dosage to be triggered.

A study conducted by EW Nutrition (Figure 1) shows an increase in intestinal lesions and blood endotoxins after a mycotoxin challenge of 200pbb of Aflatoxin B1 + 360ppb Ochratoxin in broilers at 21 days of age. The challenged birds show two times more lesions and blood endotoxins than the ones in the unchallenged control. The use of the right mitigation strategy, a product based on bentonite, yeast cell walls, and phytogenics (EW Nutrition GmbH) successfully prevented these effects as it not only mitigates mycotoxins, but also targets endotoxins in the gut.

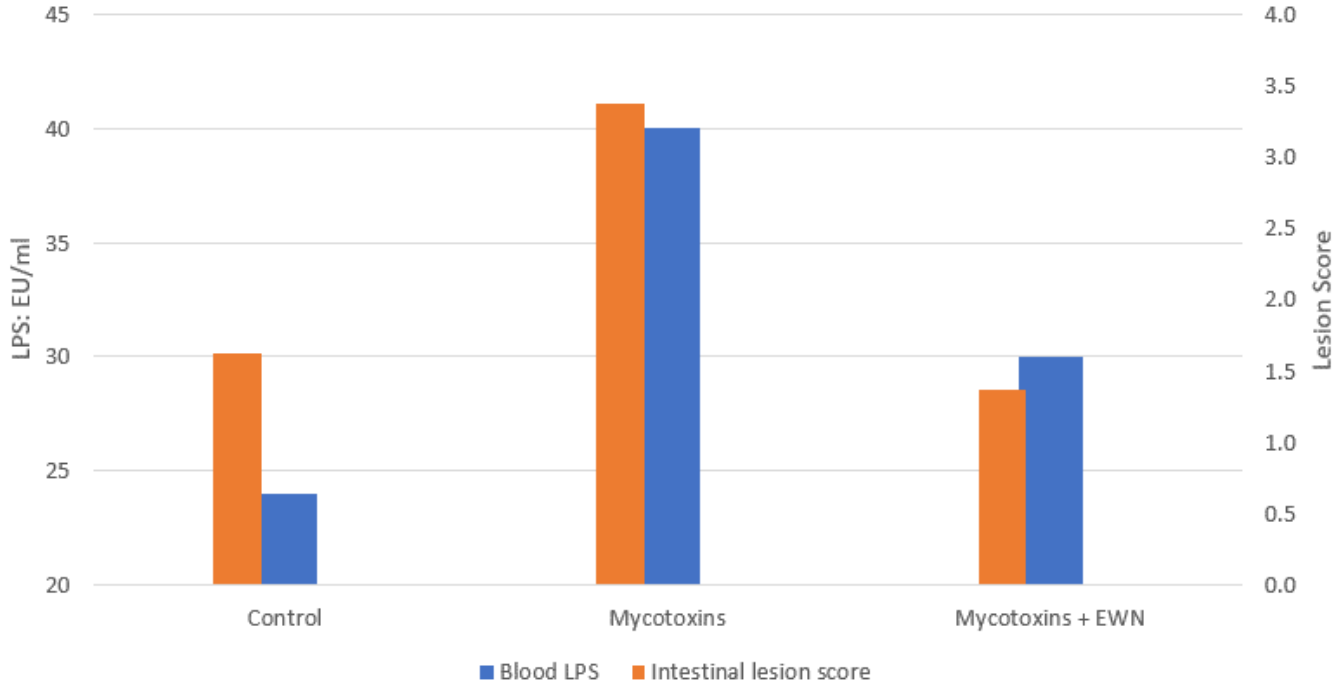


Figure 1 Blood LPS and intestinal lesion score of broilers challenged with 200ppb AFB1 + 350 ppb OTA from 1 to 21 days of age without and with an anti-toxin product from EW Nutrition GmbH (adapted from Caballero et al., 2021)

Pathogenic stress

Intestinal disease induces changes in the microbiome, reducing diversity and allowing pathogens to thrive. In clinical and subclinical necrotic enteritis (NE), the intestinal populations of GNB, including Salmonella and E.coli also increases. The lesions associated with the pathogen compromise the epithelial permeability and the intestinal barrier function, resulting in translocation of bacteria and LPS (Figure 5) into the bloodstream and internal organs.

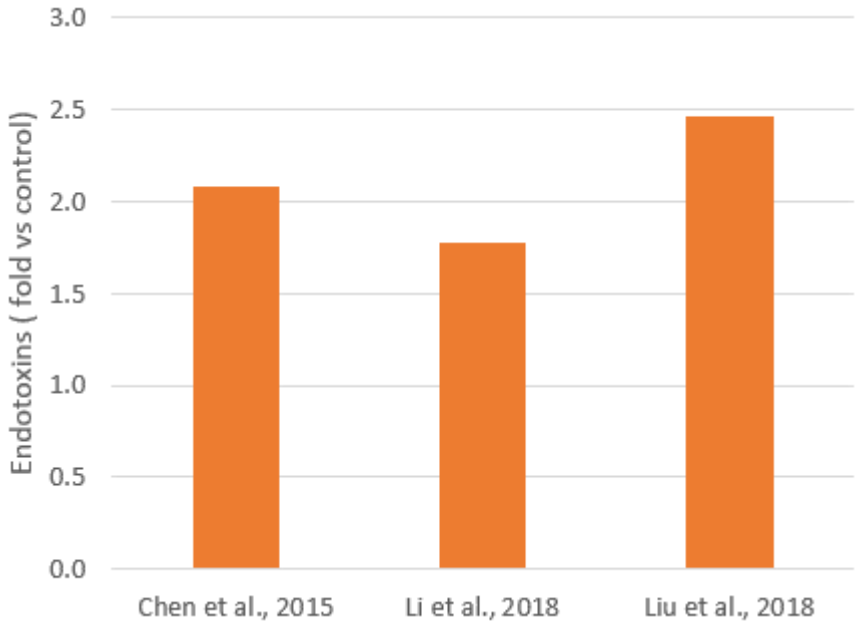


Figure 2 Increase in systemic LPS (vs a healthy control) after a NE challenge (adapted from Chen et al., 2015, Li et al., 2018 & Liu at al., 2018)

Environmental stress

Acute and chronic heat and cold stress increases gut permeability, by increasing intestinal oxidative stress and disrupting the expression of tight junction proteins. This results in the damage and destruction of intestinal cells, inflammation, and imbalance of the microbiota. An increased release and passage of endotoxins has been demonstrated in heat stress (Figure 3), as well as a higher expression of TLR-4 and inflammation.

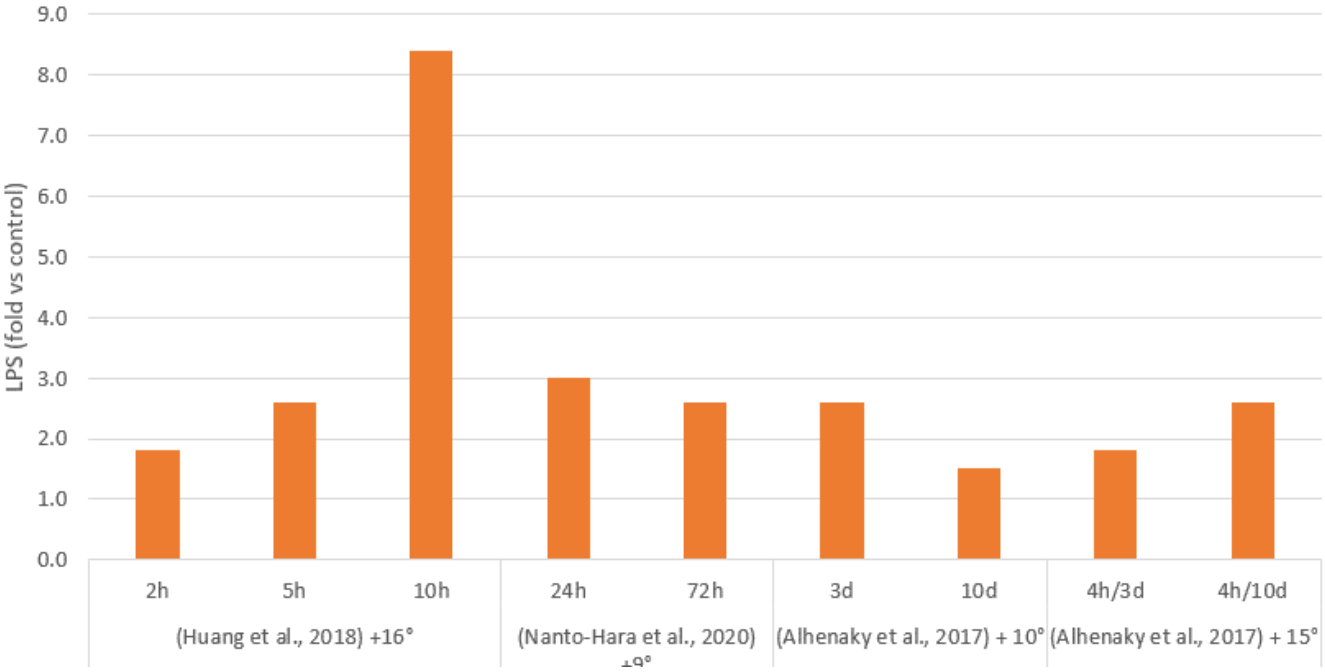
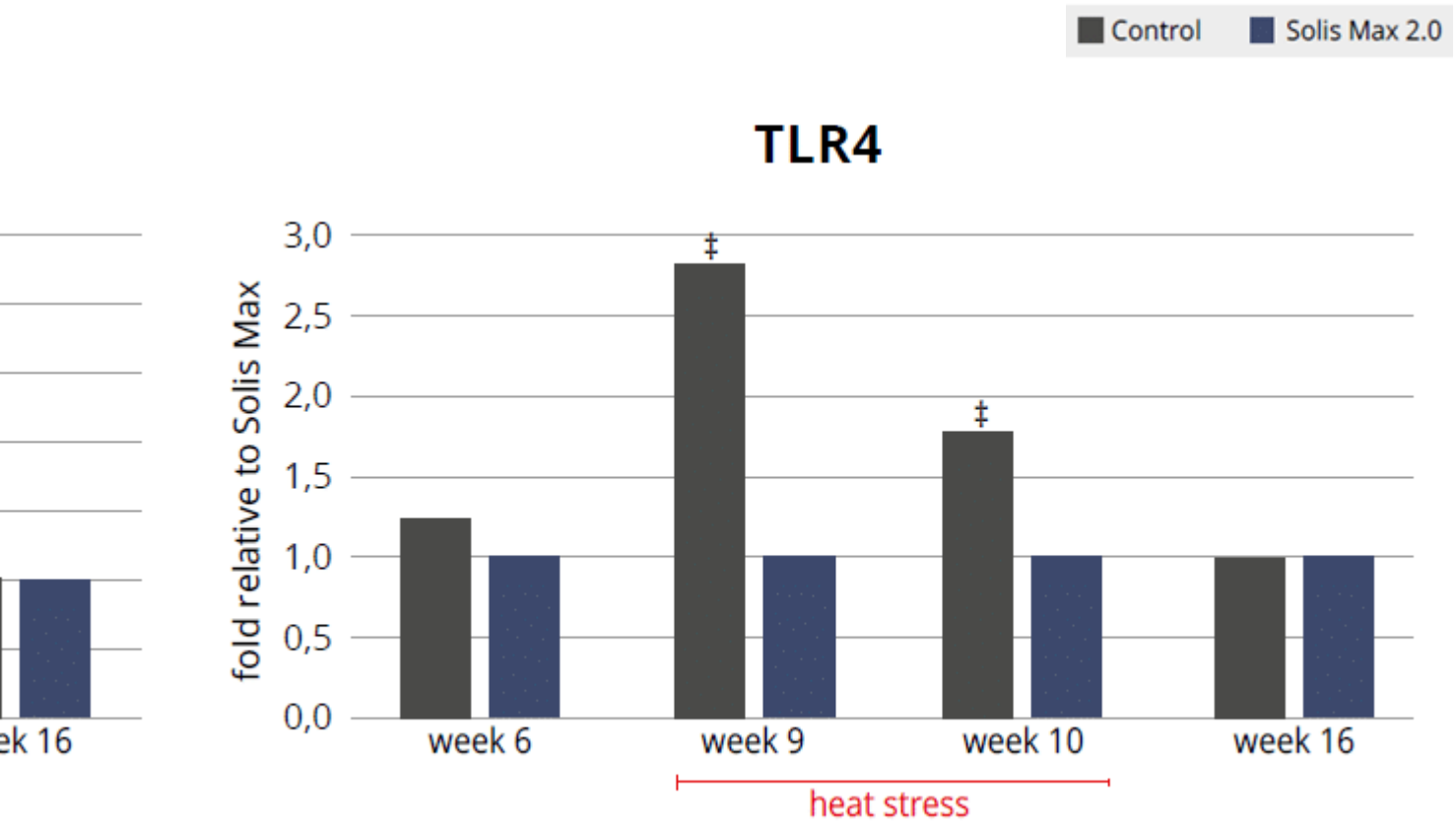


Figure 3 Systemic LPS increase (in comparison with a non-stressed control) after different heat stress challenges in broilers: 16°C increased for 2, 5 and 10 hours (Huang et al., 2018); 9°C increased for 24 and 72 hours (Nanto-Hara et al., 2020); 10°C continuously for 3 and 10 days, and 15°C 4 hours daily for 3 and 10 days (Alhenaky et al., 2017)

Zhou and collaborators (2021) showed that 72 hours of low temperature treatment in young broilers increased intestinal inflammation and expression of tight junction proteins, while higher blood endotoxins indicate a disruption of the intestinal barrier. As a consequence, the stress decreased body gain and increased the feed conversion rate.

An experiment conducted by EW Nutrition GmbH with the objective of evaluating the ability of a toxin mitigation product to ameliorate heat-stress induced LPS. For the experiment, 1760 Cobb 500 pullets were divided into two groups, and each was placed in 11 pens of 80 hens, in a single house. One of the groups received feed containing 2kg/ton of the product from the first day. From week 8 to week 12, the temperature of the house was raised 10°C for 8 hours every day.

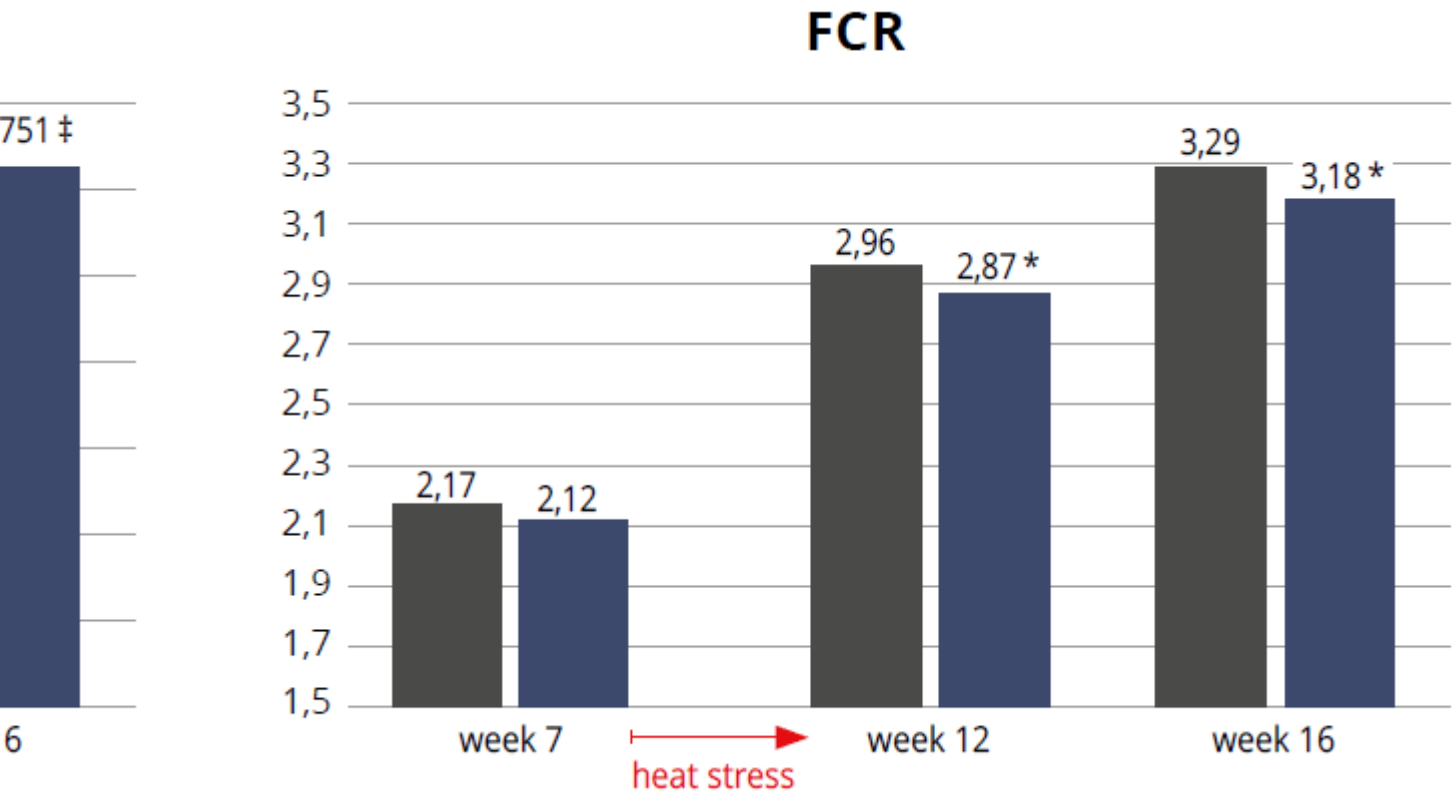
Throughout the heat stress period, blood LPS (Fig 4) was lower in the pullets receiving the product, which allowed lower inflammation, as evidenced by the lower expression of TLR4 (Fig. 5). Oxidative stress was also mitigated with the help of the combination of phytomolecules in the product, obtaining 8.5% improvement on serum total antioxidant capacity (TAC), supported by an increase in in superoxide dismutase (SOD glutathione peroxidase (GSH) and a decrease in malondialdehyde (MDH).



es of pullets before (wk 6) and during heat stress (wk 9 and 10). (*) indicates significant differences (P<0,05), and

In practice: there is no silver bullet

In commercial poultry production, a myriad stressors may occur at the same time and some factors trigger a chain of events that work to the detriment of animal health and productivity. Reducing the solution to the mitigation of LPS is a deceitfully simplistic approach. However, this should be part of a strategy to achieve better animal health and performance. In fact, EW Nutrition's toxin mitigation product alone helped the pullets to achieve 3% improvement in body weight and 9 points lower cumulative feed conversion (Figure 6).



Keeping the animals as free of stress as possible is a true priority for poultry producers, as it promotes animal health as well as the integrity and function of the intestinal barrier. Biosecurity, good environment, nutrition and good management practices are crucial; the use of feed additives to reduce the consequences of unavoidable stress also critically supports the profitability of poultry operations.

Coccidiostats in the European Union: Challenges and Perspectives

*by **Technical Team**, EW Nutrition*

Controlling coccidiosis has been and continuous to be a major concern for poultry operations. However, for decades, some of these control measures have been taking an increasingly visible toll on the overall health of the flocks, the economics of poultry production, and the environment itself. Regulations have been put in place to defend consumer health and animal welfare while maintaining profitability in poultry production.

In the European Union and elsewhere, coccidiostats or anticoccidials are an essential means of control and are categorized either as feed additives or as veterinary medicinal products. The category is dictated by the pharmacologically active substance, mode of action, pharmaceutical form, target species and route of application.

In the European Union, there are currently 11 different coccidiostats which have been granted 28 different authorizations as feed additives allowed for specific usage in chickens, turkeys, and rabbits.

Coccidiostats: the basics

Compounds designed to kill the coccidial population are known as coccidiocidal; those designed to prevent the replication and development of coccidia are known as coccidiostats. Quite often, coccidiostat or anticoccidial is the term used to describe both categories.

Coccidiostats are antimicrobial compounds which either inhibit or destroy the protozoan parasites that cause coccidiosis in livestock. Each coccidiostat has individual inhibitory mechanisms. In the case of ionophores, the compounds affect transmembrane ion transport. In the case of synthetic compounds, the molecules' mode of action is varied and, in some cases, not even entirely known (Patyra et al., 2023).

The production, manufacture, and marketing of coccidiostats, premixes with coccidiostats, and feed with coccidiostats are regulated by the Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene.

Coccidiostat categories

Coccidiostats fall under two categories:

Ionophores

Ionophores, sometimes called polyether ionophore antibiotics, are substances which contain a polyether group and are of bacterial origin. They are produced by fermentation with several strains of *Streptomyces* spp and *Actinomadura* spp. Six substances are allowed in the EU:

- monensin sodium (MON)
- lasalocid sodium (LAS)
- maduramicin ammonium (MAD)
- narasin (NAR)
- salinomycin sodium (SAL)
- semduramicin sodium (SEM)

Synthetic

Synthetic compounds include:

- decoquinate (DEC)
- diclazuril (DIC)
- halofuginone (HFG)
- nicarbazin (NIC)
- robenidine hydrochloride (ROB)

EU authorizations for ionophores are granted under specific conditions of usage, including animal category, minimum and maximum dosage, MRL (Maximum Residue Limits), and withdrawal periods.

Regulation (EC) No 1831/2003 [13] of the European Parliament and of the Council of 22 September 2003 distinguishes between coccidiostats and antibiotics used as growth promoters. Unlike the antibiotic growth promoters (forbidden in the EU since 2006), whose primary action site is the gut microflora, coccidiostats only have a secondary and residual activity against the gut microflora. That still signals that they have the potential to trigger resistance and to alter the natural balance and immune response of the farmed animals. Their potential to cause resistance has been widely acknowledged by science and practitioners alike (see below).

Why were some antimicrobial growth promoters withdrawn in 1997-1998 – but not others?

Five designated “antibiotic feed additives” were prohibited in 1997-98: Avoparcin, Bacitracin zinc, Spiramycin, Virginiamycin, and Tylosin phosphate. The EU withdrew their authorization in order to “help decrease resistance to antibiotics used in medical therapy”. The motivation specified that these antibiotics belonged to classes of compounds also used in human medicine.

On the other hand, the EU at the time allowed the remaining antibiotics for use in feed as they did not belong to classes of compounds used in human medicine. That, of course, did not mean that resistance did not develop in birds.

The Commission did acknowledge the need to phase out the remaining antibiotics. At the same time, it stated that the use of coccidiostats would not presently be ruled out “even if of antibiotic origin” (MEMO/02/66, 2022). The reason was that “hygienic precautions and adaptive husbandry measures are not sufficient to keep poultry free of coccidiosis. Modern poultry husbandry is currently only practicable if coccidiosis can be prevented by inhibiting or killing parasites during their development.”

In other words, the Commission acknowledged that the only reason ionophores were still authorized was that it believed there were no other means of controlling coccidiosis in profitable poultry production.

What issues are raised by current coccidiosis control measures?

In its 2022 Position Paper on Coccidia Control in Poultry, the European Veterinaries Federation states that “challenges in coccidia control are due to parasitic and bacterial drug (cross-)resistance. Coccidiostats also interact with other veterinary medicinal products and have a secondary residual activity against gram-positive bacteria” (FVE, 2022).

Resistance

Ever since 1939, when sulphanilamide was shown to cure coccidiosis in chickens, the industry increased the use of similar (chemical) compounds. It quickly added sulfaquinoxaline, then nitrofurazone and 3-notroroxarsone, amprolium and nicarbazin (Martins et al., 2022).

Prior to the introduction of the first ionophore, monensin, in the early 1970s, producers only had synthetic (non-ionophores) coccidiostats, characterized by rapid parasite resistance development. With the addition of ionophores, poultry operations started to rotate products between production cycles, or to use shuttle programs, with the express purpose of controlling the development of resistance. Synthetic compounds can, however, result in increased resistance in the long run (Martins et al., 2022). Moreover, studies in farmed animals indicate that sometimes even single use of antibiotics can promote the selection of resistant bacterial strains.

Another issue is the design of the rotation system, which, some researchers claim, could only delay the appearance of resistance (Daeseleire et al., 2017).

To make matters worse, for instance in the case of broilers, coccidiostats are generally administered throughout life to protect against re-infection. This may also lead to the next item on the list.

Residues

Regulation (EC) No 1831/2003 establishes Maximum Residue Limits (MRLs) for residues of an additive in relevant foodstuffs of animal origin. The goal is to control the use of coccidiostats in feed and ensure that there is no excess residue that ends up on the consumers’ plate.

Broilers can be fed with coccidiostats throughout life, with the exception of a certain withdrawal period

before slaughter. Cross-contamination of feed batches and residue formation in edible tissues of nontarget species represent valid concerns for end consumers.

Coccidiostats in food have been regulated in the Commission Regulation (EC) No 124/2009, including maximum levels for meat ranging between 2 µg/kg (monensin, salinomycin, semduramycin, and manduramycin) and 100 µg/kg (nicarbazin in liver and kidney). However, Daeseleire et al. state that "in the period 2011–14, noncompliant results were reported for maduramycin, monensin, diclazuril, lasalocid, nicarbazin, robenidine, salinomycin, narasin, semduramicin, decoquinate, halofuginone, and toltrazuril. The matrices/animals species affected were in descending order eggs, poultry, farmed game, horses, pigs, and sheep/goat (EURL workshop, 2015)". Residues in eggs are widely seen as a serious concern (Bello et al., 2023). The fact that regulations are in place constitute no safeguard against defective practices.

What alternatives to coccidiostats does the EU support?

Vaccination

Coccidiosis vaccines have been in use for the last three decades. They are based on precocious oocysts and are commonly used in breeding and laying birds, and the use in broilers is steadily increasing. There is a limited number of vaccines authorized in the EU. As vaccines are relatively costly to apply, vaccination is typically performed during 2-3 cycles only, afterwards reverting to the use of coccidiostats, which leads to a suppression of the precocious vaccine-origin strains, allowing persistent coccidiostat-resistant field strains to flourish.

Herbal products (phytomolecules)

Phytomolecules have been widely used for a variety of poultry gut health issues. Their usage in flocks at risk of coccidiosis is predicated on their ability to strengthen the natural defenses of the animal. Infection severity and consequences depend to a large extent on co-infections, gut health, and the general immunity of the bird.

Prescription veterinary medicines

Toltrazuril, amprolium, and some sulfamides (sulfamiderazin, sulfadimethoxin, trimethoprime) are used against (clinical) coccidiosis outbreaks. However, these medicines are also prone to triggering resistance and should not be widely used. Moreover, they are used when coccidiosis is already manifest on the farm, so they do not prevent economical and performance losses.

Other research

There is limited research on acidifiers, enzymes, prebiotics or probiotics acting as defenses against infection. Furthermore, oocysts are highly resistant to the common disinfectants, but there are some highly specialized types available. In general, producers are reluctant to use these methods as their benefits are limited or indemonstrable.

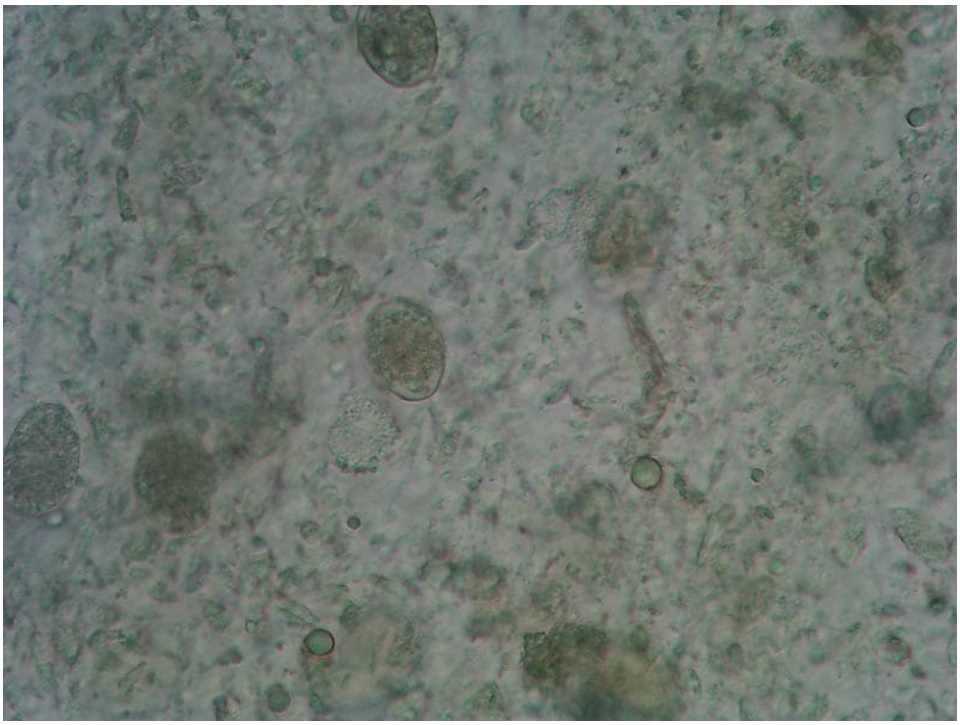
Genetic selection of the animals is also unable to offer solutions for the moment.

Ionophores as antibiotics: The U.S. case

Ionophores have demonstrated antibacterial activity (e.g., Rutkowski and Brzezinski, 2013). As opposed to their regime in the EU, where they are allowed as feed additives, in the United States, coccidiostats belonging to the polyether-ionophore class (ionophores) are not allowed in NAE (No Antibiotics Ever) and RWA (Raised Without Antibiotics) programs.

Instead of using ionophores, coccidiosis is approached by NAE/RWA US producers with a veterinary-led combination of live vaccines, synthetic compounds, phytomolecules, and farm management.

What are the perspectives of coccidiosis control?



In 2019, The European Medicines Agency (EMA) published the new Veterinary Medicinal Products Regulation (EU2019/6), emphasizing the necessity of fighting antimicrobial resistance. In response to the VMP Regulation, in November 2022, the FVE (European Veterinaries Federation) recommended tackling coccidiosis through “a combination of holistic flock health management, optimized stocking density, litter management, feeding and drinking regime as well as nutraceuticals, accompanied by appropriate biosecurity measures, vaccination and coccidiostats, where indicated”.

In its position paper, FVE advocates a “prudent and responsible use of coccidiostats”, as well as monitoring of polyether ionophores coccidiostats sales through ESVAC (European Surveillance of Veterinary Antimicrobial Consumption). European Union past experiences show that strong urges for monitoring are usually implemented and signal a need for regulation. As other countries and regions have shown excellent productivity in the absence of ionophores, it may be that, sooner or later, the EU will revise its lax attitude and embrace a stricter control of antimicrobial resistance.

FVE also recommends the development of rapid, low-cost and especially quantitative diagnostic tests for ongoing surveillance and monitoring purposes. Through fast, reliable, on-site oocyst counts, producers can cut cost and time resources and improve reaction time to preserve the health of their flocks.

From a scientific perspective, considering the range of micro-organisms affected, ionophores can be seen as antibiotics, with the usual associated risks for cross-resistance or co-selection (Wong 2019). While their current status in the European Union represents a concession to the economic security of a large and important industry, best practices in other regions show that coccidiosis can be approached holistically with solutions that reduce antimicrobial resistance and support the profitability of poultry operations.

Bio-shuttle with natural anticoccidial additives: the all-encompassing solution

As producers optimize the use of biological interventions such as vaccines, their effect on broiler performance becomes more predictable and constant.

The current common practice of rotating coccidiostats fails to take advantage of the milder precocious Eimeria population that has developed within the broiler house. Instead, the use of new, natural feed additives with anticoccidial activity that is directly related to the coccidiostat-resistant Eimeria (field) strains, as well as the precocious Eimeria strains, can help to maintain a favorable ratio between mild precocious and more virulent field strains. This can help increase the number of cycles that benefit from the vaccinations applied, even when discontinuing vaccination. Careful monitoring of oocyst shedding patterns, preferably accompanied by gut health and coccidiosis lesion scoring and performance monitoring, can guide the producer on the right time to restart vaccination and repeat the same rotation program.

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The future of coccidiosis control

*By **Madalina Diaconu**, Product Manager Pretect D, EW Nutrition and*

With costs of over 14 billion USD per year (Blake, 2020), coccidiosis is one of the most devastating enteric challenges in the poultry industry. With regard to costs, subclinical forms of coccidiosis account for the majority of production losses, as damage to intestinal cells results in lower body weight, higher feed conversion rates, lack of flock uniformity, and failures in skin pigmentation. This challenge can only be tackled, if we understand the basics of coccidiosis control in poultry and what options producers have to manage coccidiosis risks.

Current strategies show weak points

Good farm management, litter management, and coccidiosis control programs such as shuttle and rotation programs form the basis for preventing clinical coccidiosis. More successful strategies include disease monitoring, strategic use of coccidiostats, and increasingly coccidiosis vaccines. However, the intrinsic properties of coccidia make these parasites often frustrating to control. Acquired resistance to available coccidiostats is the most difficult and challenging factor to overcome.

Optimally, coccidiosis control programs are developed based on the farm history and the severity of infection. The coccidiostats traditionally used were chemicals and ionophores, with ionophores being polyether antibiotics. To prevent the development of resistance, the coccidiostats were used in shuttle or rotation programs, at which in the rotation program, the anticoccidial changes from flock to flock, and in the shuttle program within one production cycle (Chapman, 1997).

The control strategies, however, are not 100% effective. The reason for that is a lack of diversity in available drug molecules and the overuse of some molecules within programs. An additional lack of sufficient coccidiosis monitoring and rigorous financial optimization often leads to cost-saving but only marginally effective solutions. At first glance, they seem effective, but in reality, they promote resistance, the development of subclinical coccidiosis, expressed in a worsened feed conversion rate, and possibly also clinical coccidiosis.

Market requests and regulations drive coccidiosis control strategies

Changing coccidiosis control strategies has two main drivers: the global interest in mitigating antimicrobial resistance and the consumer’s demand for antibiotic-free meat production.

Authorities have left ionophores untouched

Already in the late 1990s, due to the fear of growing antimicrobial resistance, the EU withdrew the authorization for Avoparcin, Bacitracin zinc, Spiramycin, Virginiamycin, and Tylosin phosphate, typical growth promoters, to “help decrease resistance to antibiotics used in medical therapy”. However, ionophores, being also antibiotics, were left untouched: The regulation (EC) No 1831/2003 [13]of the European Parliament and the Council of 22 September 2003 clearly distinguished between coccidiostats and antibiotic growth promoters. Unlike the antibiotic growth promoters, whose primary action site is the gut microflora, coccidiostats only have a secondary and residual activity against the gut microflora. Furthermore, the Commission declared in 2022 that the use of coccidiostats would not presently be ruled out “even if of antibiotic origin” (MEMO/02/66, 2022) as “hygienic precautions and adaptive husbandry measures are not sufficient to keep poultry free of coccidiosis” and that “modern poultry husbandry is currently only practicable if coccidiosis can be prevented by inhibiting or killing parasites during their development”. In other words, the Commission acknowledged that ionophores were only still authorized because it believed there were no other means of controlling coccidiosis in profitable poultry production.

Consumer trends drove research on natural solutions

Due to consumers’ demand for antibiotic-reduced or, even better, antibiotic-free meat production, intensified industrial research to fight coccidiosis with natural solutions has shown success. Knowledge, research, and technological developments are now at the stage of offering solutions that can be an effective part of the coccidia control program and open up opportunities to make poultry production even more sustainable by reducing drug dependency.

Producers from other countries have already reacted. Different from the handling of ionophores regime in

the EU, where they are allowed as feed additives, in the United States, coccidiostats belonging to the polyether-ionophore class are not permitted in NAE (No Antibiotics Ever) and RWE (Raised Without Antibiotics) programs. Instead of using ionophores, coccidiosis is controlled with a veterinary-led combination of live vaccines, synthetic compounds, phytomolecules, and farm management. This approach can be successful, as demonstrated by the fact that over 50% of broiler meat production in the US is NAE. Another example is Australia, where the two leading retail store chains also exclude chemical coccidiostats from broiler production. In certain European countries, e.g., Norway, the focus is increasingly on banning ionophores.

The transition to natural solutions needs knowledge and finesse

In the beginning, the transition from conventional to NAE production can be difficult. There is the possibility to leave out the ionophores and manage the control program only with chemicals of different modes of action. More effective, however, is a combination of vaccination and chemicals (bio-shuttle program) or the combination of phytomolecules with vaccination and/or chemicals (Gaydos, 2022).

Coccidiosis vaccination essentials

When it is decided that natural solutions shall be used to control coccidiosis, some things about vaccination must be known:

1. There are different strains of vaccines, natural ones selected from the field and attenuated strains. The formers show medium pathogenicity and enable a controlled infection of the flock. The latter, being early mature lower pathogenicity strains, usually cause only low or no post-vaccinal reactions.
2. A coccidiosis program that includes vaccination should cover the period from the hatchery till the end of the production cycle. Perfect application of the vaccines and effective recirculation of vaccine strains amongst the broilers are only two examples of preconditions that must be fulfilled for striking success and, therefore, early and homogenous immunity of the flock.
3. Perfect handling of the vaccines is of vital importance. For that purpose, the personnel conducting the vaccinations in the hatchery or on the farms must be trained. In some situations, consistent high-quality application at the farm has shown to be challenging. As a result**,** interest in vaccine application at the hatchery is growing.

Phytochemicals are a perfect tool to complement coccidiosis control programs

As the availability of vaccines is limited and the application costs are relatively high, the industry has been researching supportive measures or products and discovered phytochemicals as the best choice. Effective phytochemical substances have antimicrobial and antiparasitic properties and enhance protective immunity in poultry infected by coccidiosis. They can be used in rotation with vaccination, to curtail vaccination reactions of (non-attenuated) wild strain vaccines, or in combination with chemical coccidiostats in a shuttle program.

In a recent review paper (El-Shall et al., 2022), natural herbal products and their extracts have been described to effectively reduce oocyst output by inhibiting Eimeria species' invasion, replication, and development in chicken gut tissues. Phenolic compounds in herbal extracts cause coccidia cell death and lower oocyst counts. Additionally, herbal additives offer benefits such as reducing intestinal lipid peroxidation, facilitating epithelial repair, and decreasing Eimeria-induced intestinal permeability.

Various phytochemical remedies are shown in this simplified adaptation of a table from El-Shall et al. (2022), indicating the effects exerted on poultry in connection to coccidia infection.

Bioactive compound	Effect
Saponins	*Inhibition of coccidia:* By binding to membrane cholesterol, the saponins disturb the lipids in the parasite cell membrane. The impact on the enzymatic activity and metabolism leads to cell death, which then induces a toxic effect in mature enterocytes in the intestinal mucosa. As a result, sporozoite-infected cells are released before the protozoa reach the merozoite phase.*Support for the chicken:* Saponins enhance non-specific immunity and increase productive performance (higher daily gain and improved FCR, lower mortality rate). They decrease fecal oocyst shedding and reduce ammonia production.
Tannins	*Inhibition of coccidia:* Tannins penetrate the coccidia oocyst wall and inactivate the endogenous enzymes responsible for sporulation.*Support for the chicken:* Additionally, they enhance anticoccidial antibodies' activity by increasing cellular and humoral immunity.
Flavonoids and terpenoids	*Inhibition of coccidia:* They inhibit the invasion and replication of different species of coccidia.*Support for the chicken:* They bind to the mannose receptor on macrophages and stimulate them to produce inflammatory cytokines such as IL-1 through IL-6 and TNF. Higher weight gain and lower fecal oocyst output are an indication of suppression of coccidiosis.
Artemisinin	*Inhibition of coccidia:* Its impact on calcium homeostasis compromises the oocyst wall formation and leads to a defective cell wall and, in the end, to the death of the oocyst. Enhancing the production of ROS directly inhibits sporulation and also wall formation and, therefore, affects the Eimeria life cycle.*Support for the chicken:* Reduction of oocyst shedding
Leaf powder of Artemisia annua	*Support for the chicken:* Protection from pathological symptoms and mortality associated with Eimeria tenella infection. Reduced lesion score and fecal oocyst output. The leaf powder was more efficient than the essential oil, which could be due to a lack of Artemisinin in the oil, and to the greater antioxidant ability of A. annua leaves than the oil.
Phenols	*Inhibition of coccidia:* Phenols change the cytoplasmic membrane's permeability for cations (H+ and K+), impairing essential processes in the cell. The resulting leakage of cellular constituents leads to water unbalance, collapse of the membrane potential, inhibition of ATP synthesis, and, finally, cell death. Due to their toxic effect on the upper layer of mature enterocytes of the intestinal mucosa, they accelerate the natural renewal process, and, therefore, sporozoite-infected cells are shed before the coccidia reaches the merozoite phase.

Table 1: Bioactive compounds and their anticoccidial effect exerted in poultry

Consumers vote for natural – phytochemicals are the solution

Due to still rising antimicrobial resistance, consumers push for meat production without antimicrobial usage. Phytomolecules, as a natural solution, create opportunities to make poultry production more sustainable by reducing dependency on harmful drugs. With their advent, there is hope that antibiotic resistance can be held in check without affecting the profitability of poultry farming.

Heat Stress in Poultry

What oxidative stress and inflammation have to do with it, why it affects gut health, and how in-feed products support mitigation strategies

Stress in animals can be defined as any factor causing disruptions to their homeostasis, their stable internal balance. Stress engenders a biological response to regain equilibrium. High environmental temperatures are among the most important environmental stressors for poultry production, causing significant economic losses for the industry.

Climate change, thermoregulation, and stress

Climate change has increased the prevalence and intensity of heat stress conditions in most poultry production areas all over the world.

The optimum temperature for poultry animals' well-being and performance –the so-called thermoneutral zone– is between 18 and 22°C. When birds are kept within this temperature range, they do not have to spend energy on maintaining constant body temperature.

Heat stress is the result of unsuccessful thermoregulation in the animals, as they produce a higher quantity of heat than they can lose. It means that there is a negative balance between the net amount of heat produced by the animal and its capacity to dissipate this body heat to the environment.

Heat stress – contributing factors

This energy imbalance is influenced by environmental factors such as sunlight, thermal irradiation, air temperature, humidity, and stocking density, but also by animal-related factors such as body weight, feather coverage and distribution, hydration status, metabolic rate, and thermoregulatory mechanisms. Moreover, stressors can be additive and different factors such as feed quality and disease can convene leading to severe losses in health and performance.

Increasing the respiratory rate -panting- is the main mechanism of chickens to loss heat, which is achieve by the evaporation of water from the respiratory tract however, relative humidity imposes a ceiling on

water evaporation and subsequent dissipation of heat. Thus, the association of heat stress not only with high temperature, but also with high relative humidity.

Heat stress can be classified into two main categories, acute and chronic:

- Acute heat stress refers to a short and fast increase in environmental temperature (a few hours), in general, poultry animals show a degree of resilience to acute heat stress.
- Chronic heat stress is when the high temperatures persist for more extended periods (several days), and their compensatory mechanisms are not sufficient to maintain tissue integrity and thus health and performance are hindered.

The animal's response to heat stress

When the environmental temperature is above the thermoneutral zone, the animals activate thermoregulation mechanisms to lose heat through behavioral, biochemical, and physiological changes and responses.

Behavioral changes

Panting and exposure of low/non-feathered body areas (raising wings) are the main behavioral mechanisms in which chickens regulate their body temperature when exposed to heat stress. These actions help the chickens to cool down, at a high toll: high energy demands, dehydration, respiratory alkalosis, lethargy, decrease in feed intake, loss of intestinal function and oxidative stress.

Physiological changes

The cardiovascular system also responds to high temperatures by deviating blood to the peripheral areas of the body to maximize the dissipation of heat. This implicates a reduced supply of nutrients and oxygen to the gastrointestinal tract, hindering its functions and provoking inflammation and oxidative stress.

The hypothalamic-pituitary-adrenal (HPA) axis gets activated, increasing the levels of circulating corticosterone, skeletal protein synthesis and the immune system is suppressed, therefore the animals stop growing and are more susceptible to disease.

Heat stress also changes the gene expression of cytokines, upregulates heat shock proteins (HSP), and reduces the concentration of thyroid hormones. When heat stress persists, these cascades of cellular reactions result in tissue damage and malfunction. The animals exposed to heat stress suffer adverse effects in terms of performance, which are widely known and include high mortality, lower growth, and production (Figure 1), and a decline in meat and egg quality.

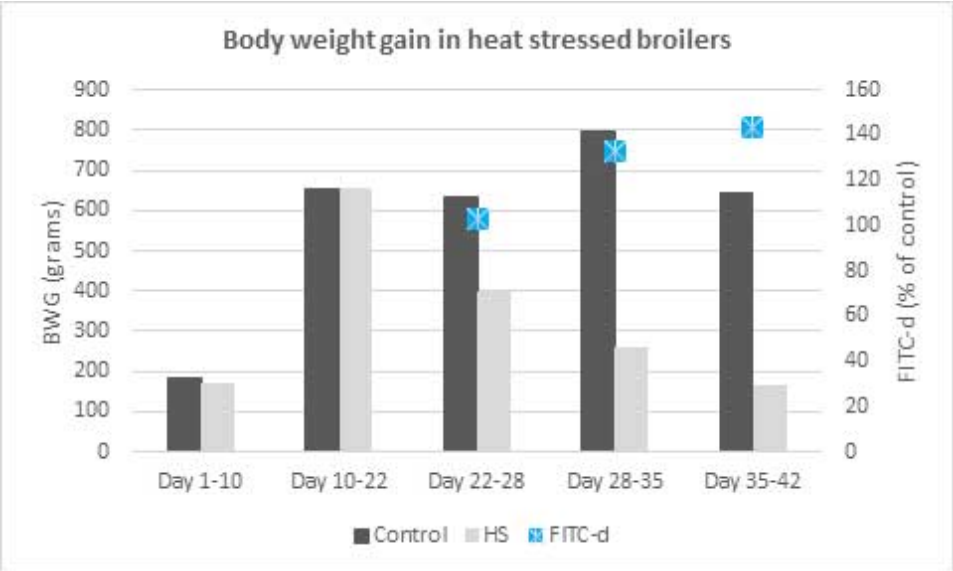

Figure 1: Body weight gain of broilers exposed to chronic heat stress (35°C continuously from day 21). A marker for tight junction permeability was added to feed (FITC-d – fluorescein isothiocyanate dextran); its fluorescence (in serum) increased with heat stress exposure time, showing higher intestinal permeability. (Adapted from Ruff et al., 2020)

Outcomes of heat stress

Oxidative stress

Oxidative stress, simply put, occurs when the amount of reactive oxygen species (ROS) and nitrogen reactive species (NRS), exceed the antioxidant capacity of the cells. Oxidative stress is regarded as one of the most critical stressors in poultry production as it is a response to diverse challenges affecting the animals.

The normal metabolism of the animal – its energy production – generates ROS and RNS, such as hydroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide. These usually are further processed by antioxidant enzymes produced by the cell, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Nutrients such as selenium and vitamins E, C, and A also participate in antioxidant processes. When the generation of ROS exceeds the capacity of the antioxidant system, oxidative stress ensues.

Heat stress leads to higher cellular energy demand, promoting an overload of ROS in the mitochondria. Consequently, oxidative stress occurs in several tissues, leading to cell apoptosis or necrosis as oxidized molecules can take electrons from other molecules, resulting in a chain reaction. Among these tissues, the gastrointestinal tract can be highly affected.

Impaired gut function

In the gastrointestinal tract, oxidative stress and the consequent tissue damage, lower feed digestion and absorption, increase intestinal permeability and modify the microbiome.

Changes in intestinal morphology and digestive function

Heat stress affects intestinal weight, length, barrier function, and microbiota, resulting in animals that have lower total and relative weight of the small intestine, with shorter jejunum and duodenum, shorter villi (Figure 2), and reduced absorption areas, in comparison to non-stressed animals.

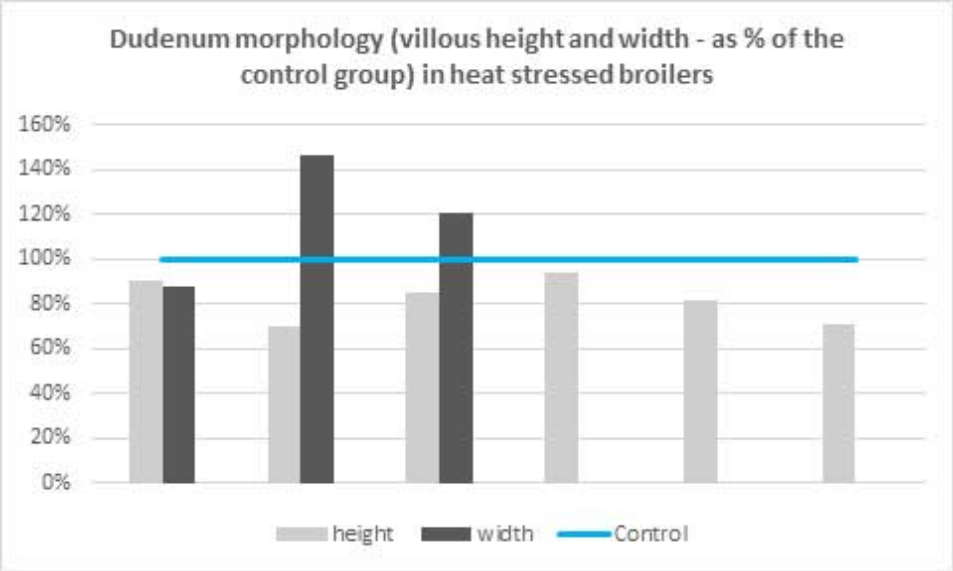

Figure 2: Villous height and width of broilers exposed to heat stress in relation with the control group (100%). Villous height is always shorter than the control group, but width can increase as the organisms shows resilience to the stressful situations and aims to recover intestinal surface. (Adapted from Jahejo et al., 2016; Santos et al., 2019; Wu et al., 2018; Abdelqader et al., 2016 ; Santos et al., 2015 and Awad et al., 2018 – by order of appearance in the graph from left to right)

Changes in the intestinal microbiome

Due to reduced feed intake and impaired intestinal function, the presence and activity

of the commensal microbiota can also be modified. Heat stress can lead to reduced populations of beneficial microbes, boost the growth of potential pathogens leading to dysbiosis and necrotic enteritis.

Changes in intestinal permeability

Several studies indicate that both acute and chronic heat stress increase gut permeability, not only by lowering feed intake, but also by increasing intestinal oxidative stress and disrupting the expression of tight junction proteins.

Heat and oxidative stress in the gut result in cell injury and apoptosis. When the tight junction barrier is compromised, luminal substances leak into the bloodstream, which constitutes the condition known as “leaky gut”. This includes the translocation of pathogenic bacteria, including zoonotic pathogens (e.g. *Salmonella* and *Campylobacter*); consequently, a higher risk of contamination of food products can be expected.

Endotoxins

Bacterial lipopolysaccharides (LPS), also known as endotoxins, constitute the main components of the outer membrane of all gram-negative bacteria and are essential for their survival. LPS have direct contact with the bacteria’s surroundings. They function as a protection mechanism against the host’s immunological response and chemical attacks from bile salts, lysozymes, or other antimicrobial agents.

Gram-negative bacteria are part of poultry animals’ microbiota; thus, there are always LPS in the intestine. Under optimal conditions, this does not affect animals because intestinal epithelial cells are not responsive to LPS when stimulated from the apical side. In stress situations, the intestinal barrier function is impaired, allowing the passage of endotoxins into the blood stream. When LPS are detected by the immune system either in the blood or in the basolateral side of the intestine, inflammation and changes in the gut epithelial structure and functionality occur.

An increased release and passage of endotoxins has been demonstrated in heat stress (Figure 3) as well as a higher expression of TLR-4 and other inflammation biomarkers, which contributes to the deleterious effects of heat stress in the animals. Moreover, blood LPS induces systemic inflammatory reactions that force the organism to divert energy to support the immune system which furthermore depresses performance.

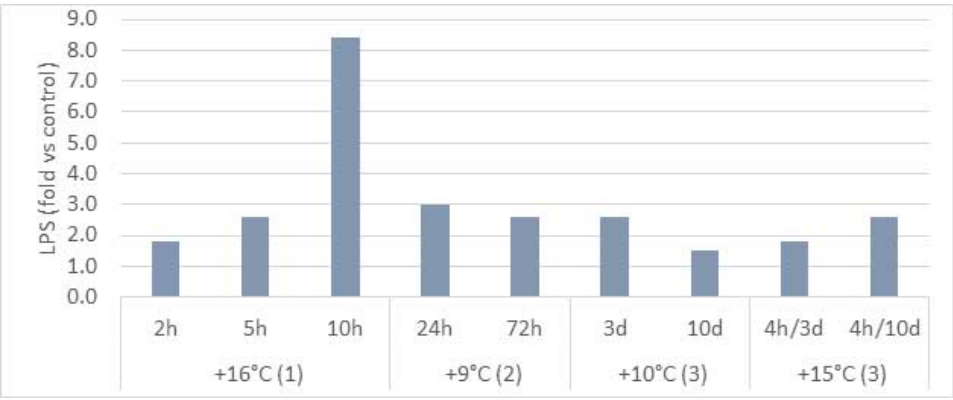

Figure 3 – Systemic LPS increase (in comparison with a non-stressed control) after different heat stress challenges in broilers:16°C increased for 2, 5 and 10 hours (Huang et al., 2018); 9°C increased for 24 and 72 hours (Nanto-Hara et al., 2020); 10°C continuously for 3 and 10 days, and 15°C 4 hours daily for 3 and 10 days (Alhenaky et al., 2017).

Mitigation strategies

Most intervention strategies deal with heat stress through a wide range of measures, including environmental management, housing design, ventilation, sprinkling, and shading, amongst others. Understanding and controlling environmental conditions is a crucial part of heat stress management.

Feed management and nutrition interventions are also recommended to reduce the effects of heat stress. They include feeding pelletized diets with increased energy coming from fats and oils, reduction of total protein with additional supplemental amino acids, increasing levels of vitamins and minerals, and adjusting the dietary electrolyte balance.

Antioxidants

Under oxidative stress conditions in the gut, there is a demand for antioxidants to counteract the excess of ROS; hence, dietary antioxidants can help reduce ROS and improve animal performance.

Research shows that certain phytomolecules, including thymol, carvacrol, cinnamaldehyde, silybinin and quercetin have antioxidant properties and improve performance under conditions of oxidative stress. The antioxidant capacity of phytomolecules manifests itself in free radical scavenging, increased production of natural antioxidants, and the activation of transcription factors. Moreover, menthol and cineol, also aid animals under heat stress by simulating the sensory cold receptors of the oral mucosa, giving the animals a cooling sensation, and reducing heat stress behavior.

Controlling LPS and oxidative stress

An experiment conducted by EW Nutrition GmbH had the objective to evaluate the ability of a product (Solis Max 2.0) in mitigating heat-stress induced LPS as well as oxidative stress.

For the experiment, Cobb 500 breeder pullets were divided in two groups, each group was placed in 11 pens of 80 hens, in a single house. One of the groups received feed containing 2kg/ton of the product from the first day. From week 8 to week 12, the temperature of the house was raised 10°C for 8 hours every day.

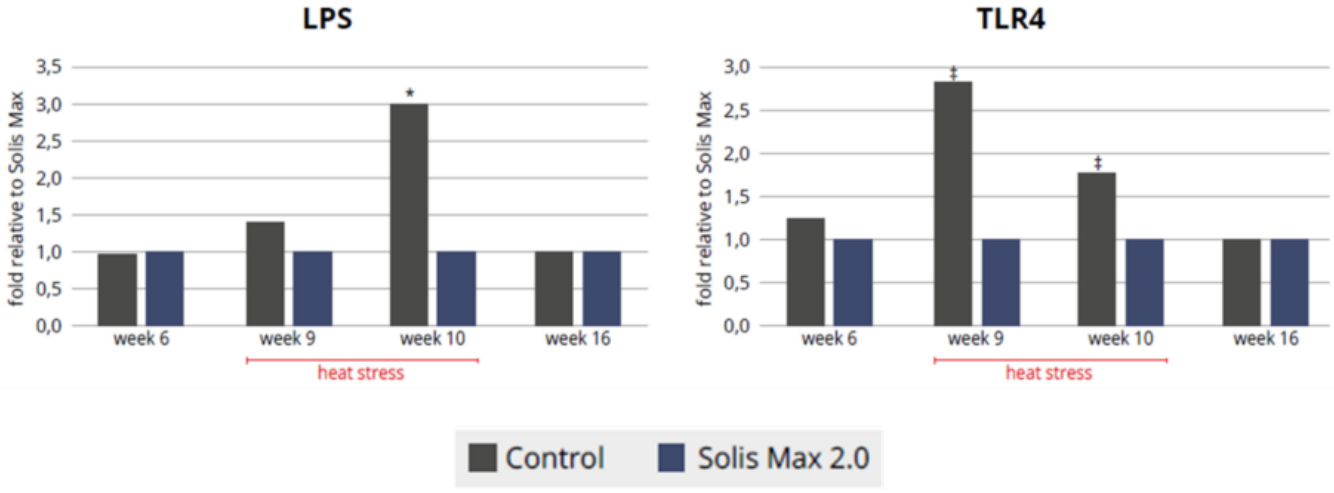

Figure 4 and 5: Blood LPS and expression of toll-like receptor 4 (TLR4) in lymphocites of pullets before (wk 6), and during heat stress (wk 9 and 10). (*) indicates significant differences (P<0,05), and (‡) a tendency to be different against the control group (P<0,1).

Throughout the heat stress period, blood LPS (Fig 4) was lower in the pullets receiving the product, which allowed lower inflammation evidenced by the lower expression of TLR4 (Fig. 5). Oxidative stress was also mitigated with the help of the combination of phytomolecules in the product (Fig. 6), obtaining 8.5% improvement on serum total antioxidant capacity (TAC), supported by an increase in in superoxide dismutase (SOD glutathione peroxidase (GSH) and a decrease in malondialdehyde (MDH).

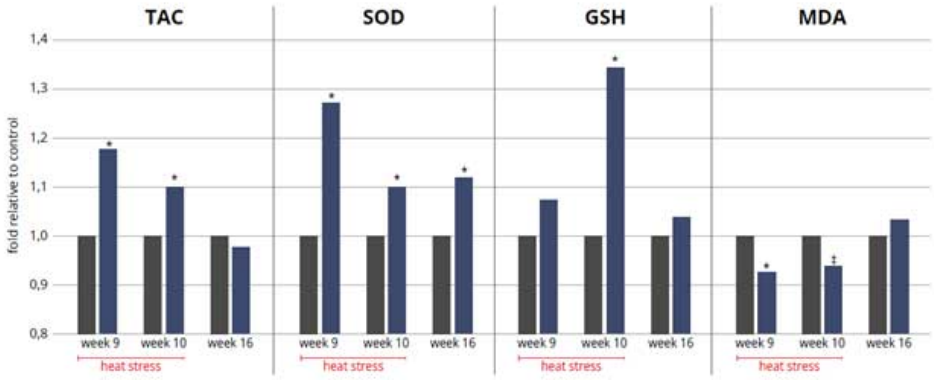

Figure 6: Antioxidant capacity of pullets during heat stress (wk 9 and 10). (*) indicates significant differences (P<0,05), and (‡) a tendency to be different against the control group (P<0,1). Parameters measured are total antioxidant capacity (TAC), super oxide dismutase (SOD), gluthatione peroxidase (GSH), and malondialdehyde (MDA).

In the bottom line, the heat stress challenge also affected performance, affecting feed conversion (9 points lower) and body weight (3% lower). The optimal supporting product was able to efficiently reduce the LPS exposure for the pullets and thus inflammation and oxidative stress were reduced, as a consequence energy could be driven to performance evidenced by a better BW and FCR.

Summary

Heat stress is a common reality in poultry production, its effects are quite complex and harmful and depend on the intensity and duration of the exposure to high temperatures.

By lowering feed digestibility, increasing gut permeability, and compromising immunity, heat stress leaves animals more susceptible to gut-health related issues such as dysbacteriosis and necrotic enteritis – and thus may increase the need to use antibiotics. Additionally, the passage of LPS through the permeable gut induces inflammation and further damage to animal welfare, health and performance.

Mitigation strategies, including support to the gut oxidative balance and lowering LPS-induced inflammation are crucial to support poultry animals in these critical periods.

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