How to develop phytogenic feed additives

By Technical Team, EW Nutrition

Modern feed additives are now commonly used as a critical tool to improve animal health. Among these, phytogenic feed additives are increasingly widely adopted. Consequently, more and more products are entering the market, leaving producers to wonder how these products differ from one another and which product performs best. To better understand the benefits that phytogenic feed additives can bring to operations, one must understand the development process feed additives undergo.

Not all feed additives are born equal

Feed additives are products that are added into an animal feed to improve its value. They are typically used to improve animal performance and welfare and consequently to optimize profitability for livestock producers.

Their purpose should not be confused with that of veterinary drugs. Feed additives provide additional benefits *beyond* the physiological needs of the animals and should be combined with other measures to improve production efficiency. Those measures include improvements in management, selection of genetics, and a constant review of biosecurity measures.

Several categories of feed additives exist. They all have in common that they are mixed into the feed or premix or the drinking water in relatively low inclusion rates to serve a *specific* purpose. Examples of feed additives are organic acids, pre- and probiotics, short and medium chained fatty acids, functional yeast products, and phytogenic feed additives. Modern feed additives also blend those different additives into combination products, increasing the value of the final products.

Phytogenic feed additives are a sub-category of additives containing phytomolecules, active ingredients which originate from plants and provide a unique set of characteristics. These molecules are produced by plants to protect themselves from molds, yeasts, bacteria, and other harmful organisms. Depending on the type of molecule, phytomolecules have different properties, ranging from antimicrobial to antioxidant and anti-inflammatory.

EW Nutrition's approach to developing Ventar D: 6 steps

The development of best-in-class phytogenic feed additives is a complex process. For Ventar D, EW Nutrition divided the process into the following steps, which can serve as a template for a successful development process:

1. Reviewing customer needs
2. Active ingredient selection
3. Technical formulation
4. Application development and scale-up
5. Performance tests
6. Safety and regulatory validation

Understanding customer needs

The most important point in developing a feed additive is customer-centricity. Understanding the challenges and needs of producers is crucial to developing feed additive solutions.

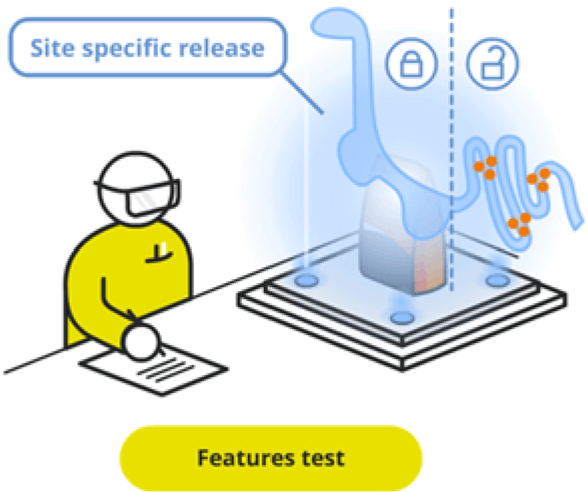
In a first step, additive producers need to evaluate and *quantify* customer needs wherever possible. This is achieved through communication and literature review: Producers, key opinion leaders, and research partners are interviewed, and their challenges are listed. In the next step, those challenges are further analyzed using scientific literature. In a final step, the customer needs are ranked according to their impact on the customer's profitability.

Subsequently, the *minimum requirements* for the new feed additive are derived. For phytogenic feed additives, this might be, for instance, something like "Improving animal performance and reducing antibiotic use while increasing profitability". The selected key performance parameters might be, for example, feed efficiency improvements in broilers.

Meeting unmet needs

Once the customer needs have been understood, the next phase of the development starts. Based on the intended mode of action, certain phytomolecules are chosen based on their described properties. In our example, this might be an antimicrobial mode of action that targets enteropathogenic bacteria in broilers, supporting gut health.

In this *in-vitro* process, the selected individual compounds will be tested for their respective antimicrobial efficacy using MIC and MBC testing. Those tests are run using high-purity compounds.

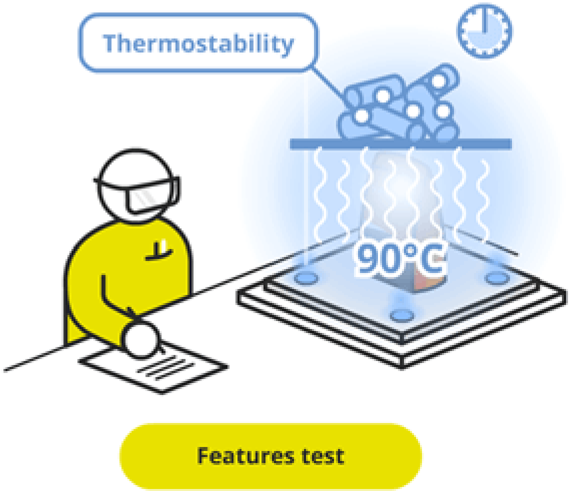


The tests will be conducted using various relevant field strains like *E. Coli, S. enterica* or *C. perfringens*. In the next step, the testing will be repeated with commercially available ingredients. The most promising compounds will be tested in more complex mixtures.

Modern phytogenic feed additives are based on the concept of combining different phytomolecules to attack bacteria in diverse ways, with their antimicrobial effects being multi-modal. This mode of action is crucial because it makes it very unlikely that bacteria can develop resistance to combinations of phytomolecules, as they do to antibiotics.

Selecting the right form of application

Feed processing is often a challenge for additives. Many phytomolecules are highly volatile and prone to volatilization and high temperatures. Especially non-protected phytogenic products are negatively affected by high pelleting temperatures and long retention times of the feed in the conditioner. The results are losses in activity.

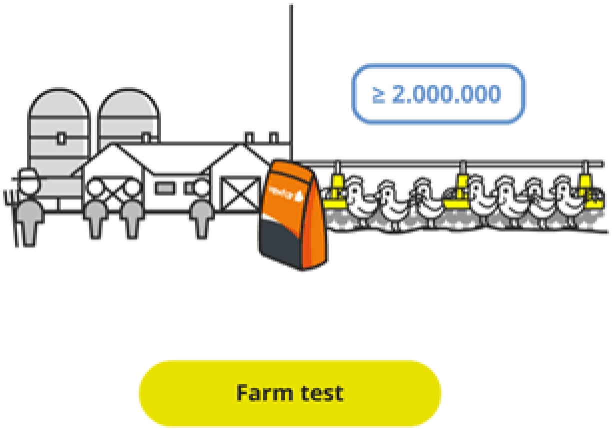


Therefore, the development of appropriate delivery systems is a preemptive method to ensure the release of the effective compounds where they should be released – in the gut of the animals. Those delivery systems can utilize emulsifiers when applying the additive via the water for drinking, or encapsulation technologies when the new additive is administered via feed.

Due to the importance of mixability, flowability, and pelleting stability for the performance of the feed additives, the exact types of emulsifiers, carrier, and technologies used in their production is often considered corporate intellectual property.

The importance of *in-vivo* evaluations

In one of the last steps of the development, the newly developed feed additive prototype needs to prove its safety and efficacy in the animal. Hence the need to run evaluation studies to confirm the mode of action chosen in the initial lab phase. Typically, the additive will be tested in the target species in in-house and external research institutes.



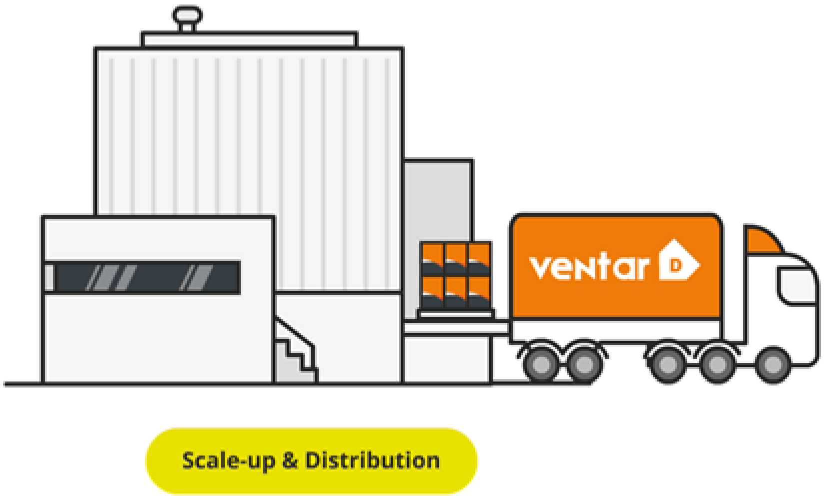
For a phytogenic feed additive, that might entail comparing its effect on body weight gain, feed efficacy, and gut health against different control groups. Additionally, the newly developed feed additive might be compared to existing additives to get a better understanding of its capabilities.

Dose-finding studies are conducted to verify the chosen dose recommendation and additional overdosing studies are conducted to prove the safety of the additive for both animals and consumers. In certain markets or regulatory environments, additional studies might be required. Those can contain environmental safety assessments or proof that the new additive does not create residues in animal products.

Case study: Ventar D

For Ventar D, the process followed these steps meticulously, in agile iterative development loops that went from the customer need to formulation, testing, scale-up, in-house and external trials, and finally production.

These steps ensured that the final product that reaches the customer's doorstep delivers on the expectations – and more.



Choose your phytogenic products wisely

The plethora of (phytogenic) feed additives in the market leaves producers with many options to choose from. However, only scientifically developed feed additives can be relied upon to optimize both animal health and production profitability. It is important to select reliable feed additive producers who developed their phytogenic product with the customers' challenges in mind and went through all the steps necessary to create a high-performing and safe additive.

EW Nutrition launches Pretect D to support poultry gut health during challenging periods

VISBEK, 28 September – EW Nutrition announces the launch of a novel gut health solution for poultry. Pretect D, a proprietary blend of phytomolecules, helps maintain bird performance and farm profitability.

Trials indicate that Pretect D offers natural support even during *Eimeria*-related challenges, making it an effective addition to programs focused on gut health issues.

“EW Nutrition is a front runner when it comes to innovations driving lower use of antibiotics and harmful chemicals in the animal production industry,” says Michael Gerrits, Managing Director. “The introduction of Pretect D signifies our commitment to helping customers make livestock production more sustainable through best-in-class natural solutions.”

Research with Pretect D conducted around the globe, in research institutes and under commercial conditions, evidenced improved body weight and lower feed conversion rate. EW Nutrition is also following up on initial results indicating significant oocyst count reduction.

“Poultry producers are affected by reduced animal performance and high costs for preventive and therapeutic control,” says Madalina Diaconu, Product Manager for Pretect D. “What our product brings to the market is an ability to support the natural defenses of birds. We’re also investigating our product’s ability to impair the growth cycle of the *Eimeria* population.” Pretect D is developed to be used in combination with vaccines, ionophores and chemicals, as part of the shuttle or rotation program.

About EW Nutrition

For the global animal production and feed industries, EW Nutrition offers innovative, comprehensive solutions for gut health, feed quality, pigmentation, digestibility, on-farm performance and more.

Headquartered in Germany, with R&D and manufacturing facilities around the world, EW Nutrition owns the entire value chain, from development and scale-up to production, distribution, and support in 90+ markets.

Want better poultry performance? Focus on gut health

by **Ruturaj Patil**, Product Manager Phytogenic Liquids, EW Nutrition

Commercial poultry operations have undergone enormous changes in production practices over the last 50 years. Genetic selection for high production rates, along with upgraded management techniques and dietary measures, have led to increased performance standards in all poultry operations (Kogut et al., 2017). However, it is sensible to now look into whether poultry performance may soon reach a ceiling due to genetic and/or physiological limits. So, aiming at further performance optimization, poultry researchers and producers are now focusing on gut health.

LOWRES IMG

Gut health management is key to sustainably improve poultry performance

The caveat, of course, is that, due to concerns about antimicrobial resistance, antimicrobial growth promoters (AGPs) no longer offer the easy answer to gut health issues they once were. To preserve antibiotics' efficacy for cases where they are indispensable, gut health-oriented performance enhancement needs to come from other sources. This article reviews the principles of gut health management in poultry and shows how Activo liquid, a phytomolecules-based in-water solution, strengthens poultry performance by targeting gut health.

Gut health: the cradle of poultry performance

Gastrointestinal health in poultry birds encompasses three dimensions: microflora balance, gut structural integrity, and immune system status. The gut plays a vital and diverse role as it hosts most microorganisms in the body, contains more than twenty different hormones, digests and absorbs the nutrients, and accounts for 20% of body energy expenditure (Choct, 2021). When gut health is compromised, digestion and nutrient absorption are affected, with likely detrimental effects on feed conversion, followed by economic loss and greater disease susceptibility. Disease resistance and nutrient utilization largely depend on maintaining a beneficial gut antioxidant status, improving gut integrity, and modulating the gut microbiota (Oviedo-Rondón, 2019).

In birds, the gut is separated into five distinct regions (Figure 1): crop, proventriculus, gizzard, small intestine (duodenum, jejunum, and ileum), and large intestine (ceca, cloaca, and vent). Each of these regions has a specific role in the secretion of digestive juices and enzymes, the grinding of feed particles and then the digestion and absorption of nutrients (Bailey 2019).

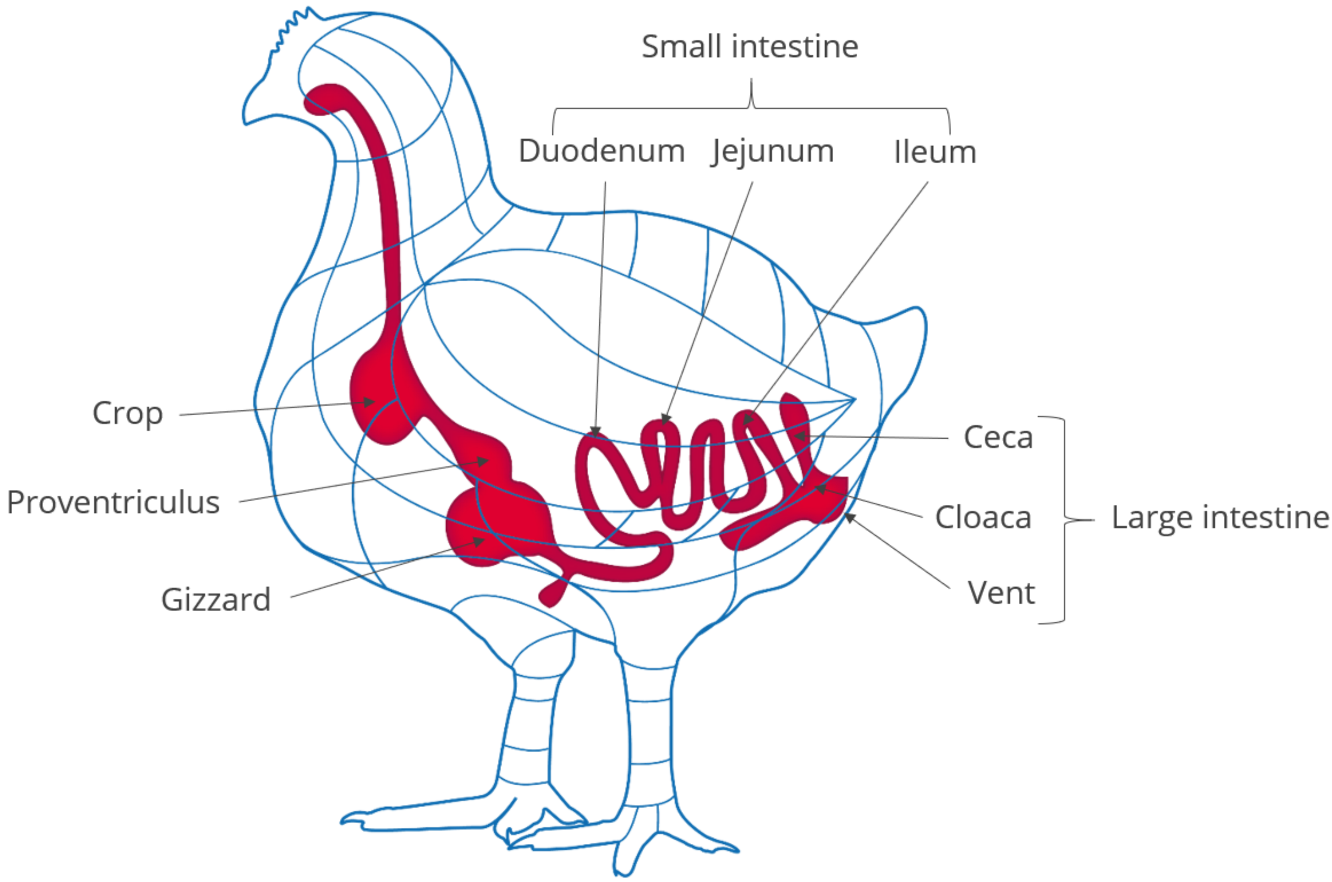


Figure 1: Schematic overview of poultry gastrointestinal tract

Factors affecting gut health

Gut health is influenced by the balance between the physiological health status of host, the gut microbiota, and a range of specific factors, all of which producers need to consider. From a management perspective, key factors encompass deprived gut health, biosecurity, and production stress, which is elevated during certain critical stages (see table 1). Environmental factors include humidity, temperature, and ventilation. Dietary factors, such as feed and water quality, feed composition, and mycotoxin contamination, also impact the development and ongoing state of poultry birds' intestinal microbiota.

Critical stages		**Signs and symptoms**
Broilers • Vaccination • Feed change • Heat/cold stress • Finisher stage	Layers/breeders • Vaccination • Feed change • Transition from grower to laying house • Point of lay to peak production • Late laying phase	• Diarrhea • Undigested feed in feces • Subclinical necrotic enteritis / cocci • Wet litter condition / food pad lesions • Increased mortality

Table 1: Critical stages for gut health issues in poultry birds

The future is here: antibiotic reduction through improved gut health

There is a strong trend towards antibiotic-free (ABF) poultry production, fueled by AGP bans in certain regions (such as the European Union) and increasing consumer interest in avoiding products containing traces of AGPs. ABF systems can be profitable as long as the prices for the final ABF products can cover the investment costs necessary to produce these products. Larger-scale, sustainable ABF production will depend on developing a more profound understanding of intestinal health alongside the development of practical applications that foster gut health throughout each step of the production system.

Feed additive solutions to support birds during challenging situations

Feed additive manufacturers are looking into accessible alternatives to mitigate the need for antibiotics in ABF systems, requiring enormous research and development efforts. At EW Nutrition, our approach is to offer a holistic antibiotic reduction program for gut health management in poultry. The program comprises feed- and water-based solutions to support gut health during high-challenge periods. Activo liquid, an in-water solution containing standardized amounts of selected phytomolecules, is a key component of our program. Based on its three-fold mode of action, Activo liquid provides gut health support that improves livability and feed efficiency:

- **Antimicrobial** activity hinders the growth of potential pathogens
- **Better gut integrity and positive microbiota** optimize feed efficiency and gut health
- **Antioxidant** activity at the gut level prevent free radical formation and oxidative stress

As a water-based solution, Activo liquid provides a quick and flexible option for gut health control on poultry farms. The benefits of Activo liquid supplementation have been demonstrated through several scientific and field studies globally.

Activo liquid reduces mortality and improves feed conversion in broilers

Numerous field studies for antibiotic-free broilers across different countries and breeds show: on average, the inclusion of Activo liquid reduces mortality by 0.6% and improves FCR by 5%, compared to non-supplemented control groups (Figure 2).

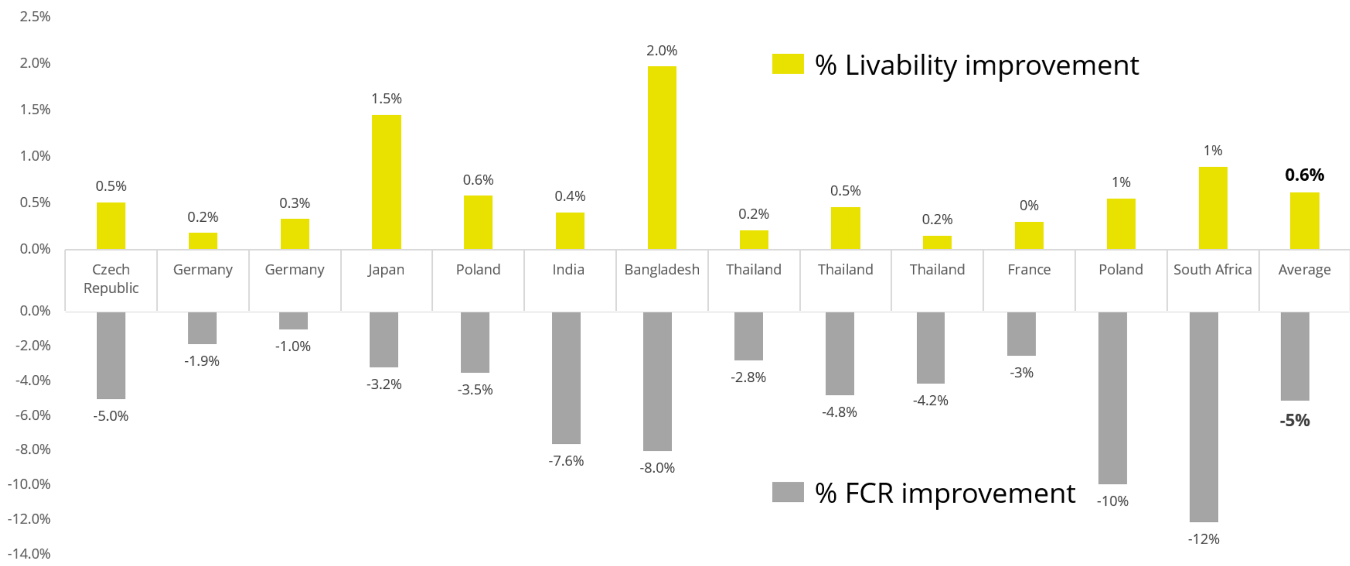


Figure 2: Changes in livability and feed conversion rate in Activo liquid-supplemented broilers

Activo Liquid supports broiler breeders from start of lay to pre-peak production

Broiler breeders are prone to gut-related issues from the start of lay to pre-peak production (age 24 to 32 weeks). This period is characterized by sudden changes in feed consumption and high production stress. Field studies from Thailand show that Activo liquid supplementation in this phase leads to improved livability and higher laying rates.

A of 34,000 female broiler breeders during the first 9 weeks of production found that for the group receiving Activo Liquid (200 ml / 1000 L, 5 days per week, 6 hours per day):

- The average laying rate/HH increased by 7.2 % during the trial period,
- Nearly 3 more hatching eggs per hen housed and about 5 more hatching eggs than the genetic standard were produced, and
- Mortality decreased by 0.2 % points compared to the control.

Another study, again evaluating the first 9 weeks of production using 20,000 birds, also found that broiler breeders supplemented with Activo Liquid show reduced mortality, a higher laying rate, and more hatching eggs per hen housed (Figure 3).

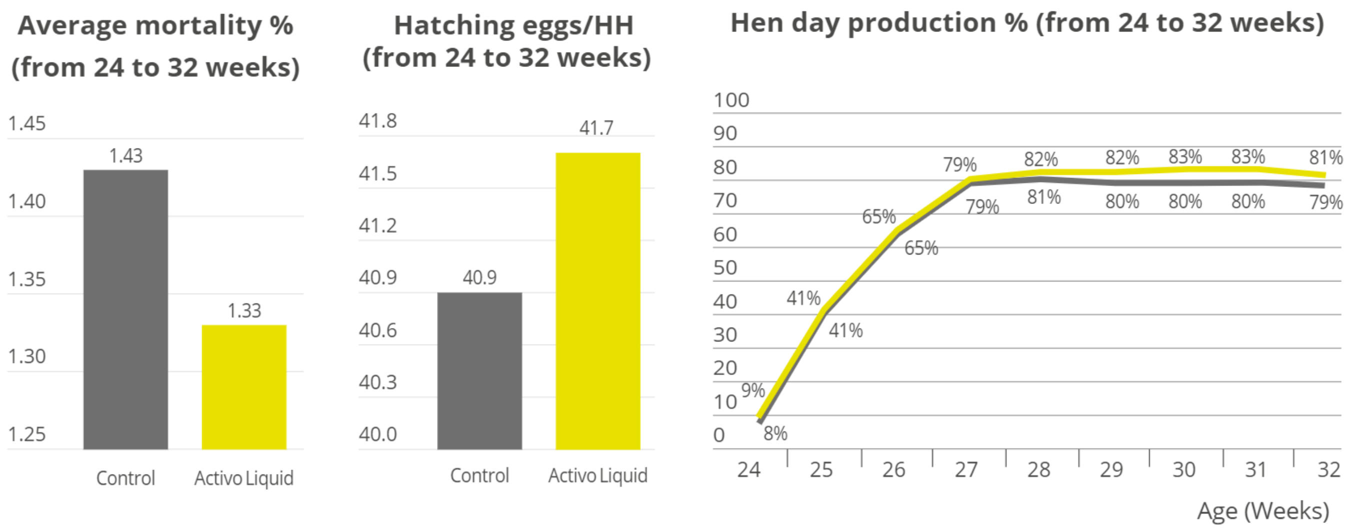


Figure 3: Performance results from Cobb broiler breeders, Activo liquid supplementation vs. control

Activo program improves layer productivity

Commercial layers often becomes challenged due to stress originating from management issues, gut pathogens, and an improper assimilation of nutrients. The negative impact on gut health can result in poor uniformity, low livability, and impaired body weight gain. The Activo program (a combination of Activo powder and liquid) has been found to improve layer performance, likely because its phytogenic components foster better intestinal integrity and microbiome diversity.

A study of 8 replicates with 36 Hy-line brown laying hens was conducted in China, for instance, testing the inclusion of both Activo (100 g / MT of feed) and Activo Liquid (250 ml / 1000 L for 4 days, every 2 weeks, from week 15 to week 25). It found that the Activo program can effectively support the animals in coping with NSP-rich diets (Figure 4). Supplemented layers showed 3.36% higher egg production, representing more than 3.5 eggs and more than 150 grams of additional egg mass per hen housed during the period. Better gut health in the Activo Program gut was evidenced by a better hen body weight , as well as higher yolk color, lower FCR, and improved intestinal morphology parameters.

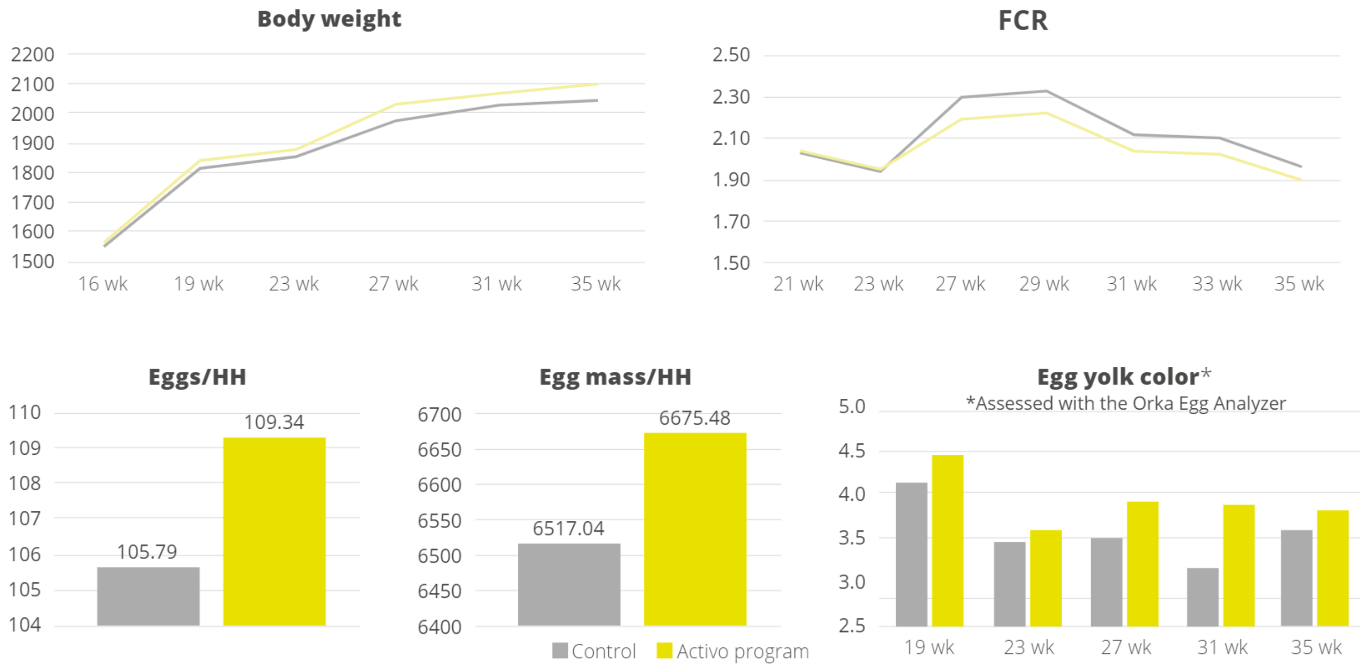


Figure 4: Performance results from Hy-line layers, Activo program vs. control

Conclusion: future improvements in poultry performance will come from the gut

As the trend towards ABF poultry production gains momentum, a concerted focus on supporting birds' gut health is key to achieving optimal performance. Multiple field studies of Activo liquid application demonstrate that, due to their antimicrobial and antioxidant properties, the phytomolecules present in Activo liquid effectively support birds' intestinal health during challenging periods.

In combination with good dietary, hygiene and management practices, phytomolecules offer a potent tool for reducing the use of antibiotics. The inclusion of Activo liquid in their birds' diets allows poultry producers to achieve better gut health and, thus, stronger performance results in a sustainable way.

References

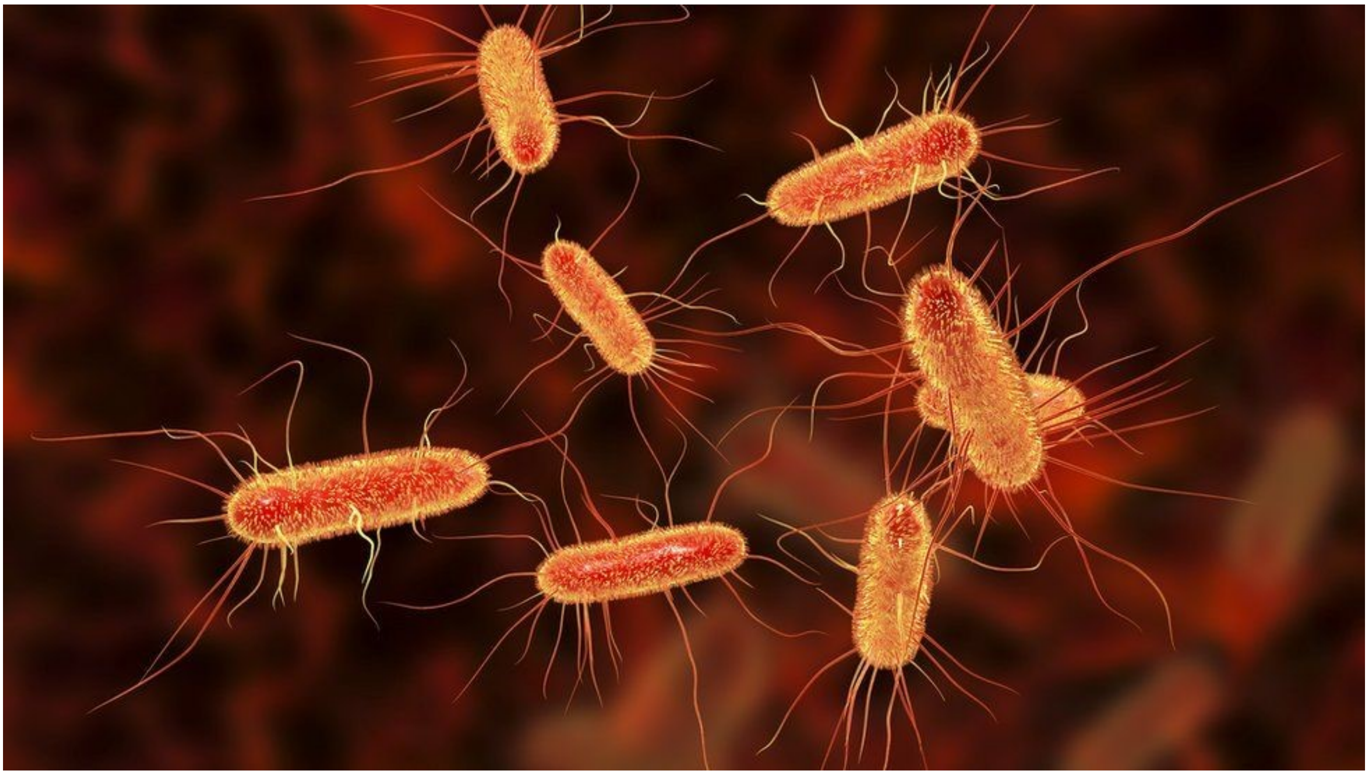
Bailey, Richard A. "Gut Health in Poultry: the World within – Update." The Poultry Site, July 6, 2021. https://www.thepoultrysite.com/articles/gut-health-in-poultry-the-world-within-1.

Choct, Mingan. "The Importance of Managing Gut Health in Poultry." Poultry Hub Australia, November 26, 2014. https://www.poultryhub.org/importance-managing-gut-health-poultry.

Kogut, Michael H., Xiaonan Yin, Jianmin Yuan, and Leon Bloom. "Gut Health in Poultry." CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 12, no. 031 (October 1, 2017): 1–7. https://doi.org/10.1079/pavsnnr201712031.

Oviedo-Rondón, Edgar O. "Holistic View of Intestinal Health in Poultry." Animal Feed Science and Technology 250 (2019): 1–8. https://doi.org/10.1016/j.anifeedsci.2019.01.009.

The hidden danger of endotoxins in animal production



by Technical Team, EW Nutrition

Find out why LPS can cause endotoxemia and how intelligent toxin mitigation solutions can support endotoxin management.

Each E. coli bacterium contains about 100 lipopolysaccharides molecules in its outer membrane

Lipopolysaccharides (LPS) are the major building blocks of the outer walls of Gram-negative bacteria. Throughout its life cycle, a bacterium releases these molecules, which are also known as endotoxins, upon cell death and lysis. The quantity of LPS present in Gram-negative bacteria varies between species and serotypes; *Escherichia coli*, for example, contain about 100 LPS/bacterial cell. When these are released into the intestinal lumen of chickens or swine, or in the rumen of polygastric animals, they can cause serious damage to the animal's health and performance by over-stimulating their immune system.

How lipopolysaccharides cause disease

LPS are rather large and structured chemical molecules with a weight of over 100,000 D. They are highly thermostable; boiling in water at 100°C for 30 minutes does not destabilize their structure. LPS consist of three chemically distinct sections: a) the innermost part, lipid A, consisting mostly of fatty acids; b) the core, which contains an oligosaccharide; and c) the outer section, a chain of polysaccharides called O-antigen (Figure 1).

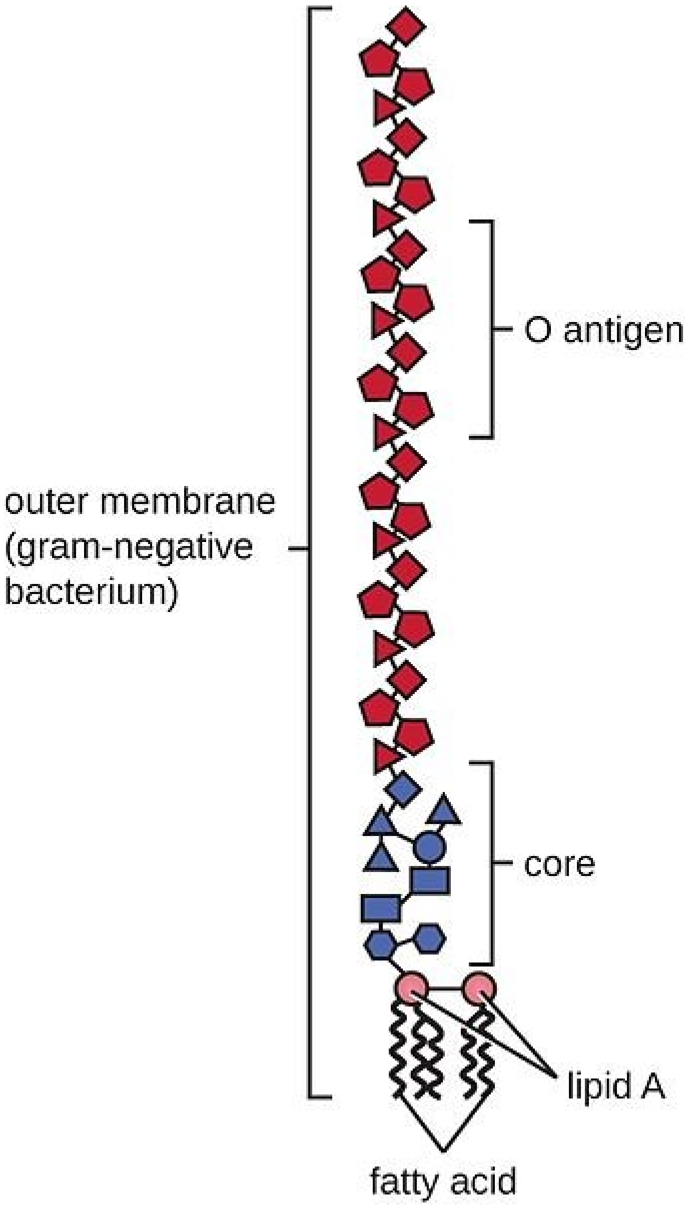


Figure 1: Structure of an LPS

The toxicity of LPS is mainly caused by lipid A; however, both lipid A and O-antigen stimulate the immune system. This happens when the LPS pass the mucosa and enter the bloodstream or when they attack the leukocytes.

The intestinal mucosa is the physical immune barrier that protects the microvilli from external agents (bacteria, free LPS viruses, etc.). Despite its strength (the thickness, for example, amounts to ≈830 µm in the colon and ≈123 µm in the jejunum), vulnerable points exist (cf. Zachary 2017).

LPS can easily come into contact with the cells of the *lamina propria* (a layer of connective tissue underneath the epithelium) through the microfold (M) cells of the Peyer's patches (which consist of gut-associated lymphoid tissue). The M cells are not covered by mucus and thus exposed.

Secondly, LPS can also pass through the mucosa, where they become entangled in this gelatinous structure. There, they come into contact with the lymphocytes or can reach the regional lymph nodes through the afferent lymphatic vessels.

Thirdly, LPS might affect the tight junctions, the multiprotein complexes that keep the enterocytes (cells that form the intestinal villi) cohesive. By destabilizing the protein structures and triggering enzymatic reactions that chemically degrade them, LPS can break the tight junctions, reaching the first capillaries and, consequently, the bloodstream.

The presence of endotoxins in the blood, endotoxemia, can trigger problematic immune responses in animals. An innate immune stimulation leads to an increase in the concentration of pro-inflammatory cytokines in the blood and, consequently, to an induced febrile response in the animal: heat production increases, while the available metabolic energy decreases. As a result, performance suffers, and in the worst-case scenario, septic shock sets in. Furthermore, when LPS compromise intestinal integrity, the risk of secondary infections increases, and production performance may decline.

LPS’ modes of action

How does all of this happen? The physiological consequences of endotoxemia are quite complex. Simplified, the immune system response to LPS in the blood takes three forms:

- The stimulation of **TLR4** (toll-like receptor 4) induces monocytes and macrophages to secrete critical pro-inflammatory cytokines, primarily interleukin (IL) IL-1β, IL-6, IL-8, and tumor necrotic factor (TNF) α and β. TLR4 is a structure on the cell membrane of mainly macrophages and leukocytes, which is activated by the LPS-binding protein (LBP).
- The **complement cascade** constitutes about 10% of plasma proteins and determines the chemotaxis and activation of leukocytes. It can form a membrane attack complex (MAC), which perforates the membranes of pathogenic cells, enabling lysis.
- The **Hagemann factor**, also known as coagulation factor XII: once stimulated by LPS, it initiates the formation of fibrin (through the intrinsic coagulation pathway), which might lead to thrombosis. The Hagemann factor directly stimulates the transformation of prekallikrein to kallikrein (enzymes involved in regulating blood pressure).

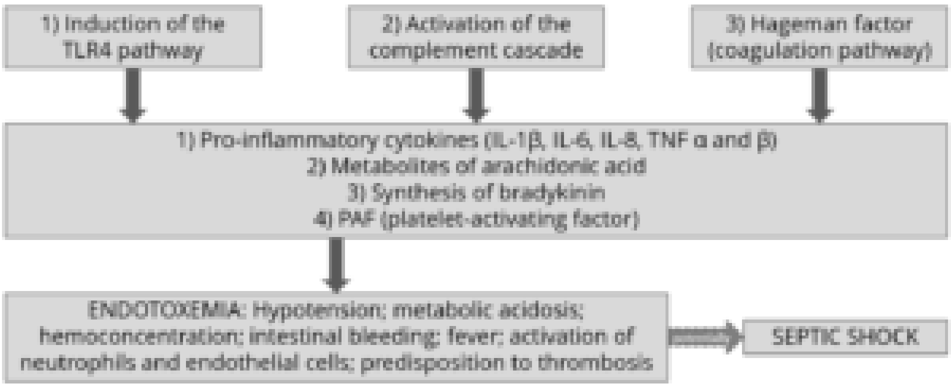


Figure 2: How LPS leads to endotoxemia – 3 modes of action

These three modes of action of inflammatory stimulation lead to important physiological reactions:

- **Pro-inflammatory cytokines** (see above) modulate the functional expression of other immune cell types during the inflammatory response;
- **Metabolites of arachidonic acid** (prostaglandins, leukotrienes, and lipoxins), intra- and extracellular messengers that influence the coagulation cascade;
- Synthesis in the blood of **bradykinin**, a peptide responsible for the typical symptoms of inflammation, such as swelling, redness, heat and pain;
- **PAF** (platelet-activating factor), which creates inflammatory effects through narrowing of the blood vessels and constriction of the airways, but also through the degranulation of leukocytes.

The symptoms of endotoxemia are: hypotension, metabolic acidosis, hemoconcentration, intestinal hemorrhage, fever, activations of neutrophils and endothelial cells, and predisposition to thrombosis.

In case of a progression to septic shock, the following sequence takes place:

1) Reduction in blood pressure and increased heart rate (hemodynamic alterations)

2) Abnormalities in body temperature

3) Progressive hypoperfusion at the level of the microvascular system

4) Hypoxic damage to susceptible cells

Up to here, symptoms follow a (severe) endotoxemia pathogenesis. A septic shock furthermore entails:

5) Quantitative changes in blood levels of leukocytes and platelets

6) Disseminated intravascular coagulation (see Hageman factor)

7) Multi-organ failure

8) Death of animal

If an animal is continously challenged with endotoxins, experiences septic shock, or comes close to it, it risks developing LPS tolerance, also known as CARS (compensatory anti-inflammatory response syndrome). This syndrome essentially depresses the immune system to control its activity. The anti-inflammatory prerogative of CARS is not to interfere directly with the elimination of pathogens but to regulate the “excessive” inflammatory reaction in a hemostatic way. However, this regulation can be extremely dangerous as the syndrome involves a lack of homeostasis control, and an excessive depression of the immune system leaves the organism exposed to the actual pathogens.

Farm animal research on endotoxemia pathogenesis

Lipopolysaccharides are difficult to quantify in the intestine of a live animal. One way to evaluate a possible endotoxemia is to analyze biomarkers present in the bloodstream. The most important one is the LPS themselves, which can be detected in a blood sample taken from the animal via ELISA. Other biomarkers include pro-inflammatory interleukins, such as TNF α and β, IL-6 or IL-8, and fibrin and fibrinogen (though they are not specific to endotoxemia). It is vital to carry out a blood sample analysis to deduce a possible endotoxemia from symptoms and performance losses in the animal.

How the metabolic effects of endotoxemia depress performance

One of the biggest issues caused by endotoxemia is that animals reduce their feed intake and show a poor feed conversion rate (FCR). Why does this happen? The productive performance of farm animals (producing milk, eggs, or meat) requires energy. An animal also requires a certain baseline amount of energy for maintenance, that is, for all activities related to its survival. As a result of inflammation and all those physiological reactions mentioned above, endotoxemia leads to a feverish state. Maintenance needs to continue; hence, the energy required for producing heat will be diverted from the energy usually spent on producing milk, egg, meat, etc., and performance suffers.

The inflammation response can result in mitochondrial injury to the intestinal cells, which alter the cellular energy metabolism. This is reflected in changes to the levels in adenosine triphosphate (ATP), the energy “currency” of living cells. A study by Li et al. (2015) observed a respective reduction of 15% and 55% in the ATP levels of the jejunum and ileum of LPS-challenged broilers, compared to the unchallenged control group. This illustrates the extent to which animals lose energy while they experience (more or less severe) endotoxemia.

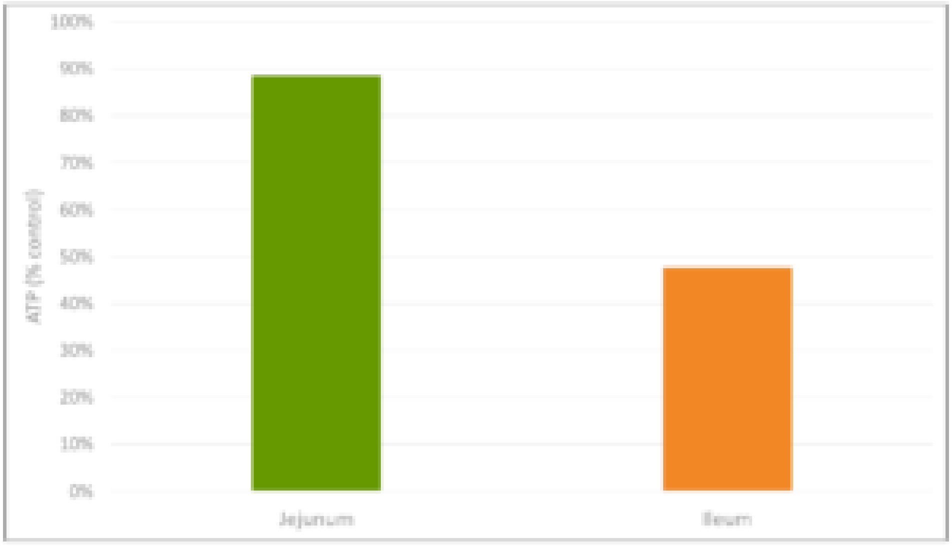


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from [Li et al., 2015](#))

A [piglet study by Huntley, Nyachoti, and Patience (2017)](#) took this idea further (Figure 4): 3 groups of 10 Yorkshire x Landrace pigs, weighing between 11 and 25 kg, were studied in metabolic cages and in respiratory chambers. This methodology allows for simultaneous measurement of oxygen consumption, CO_2 production, energy expenditure, physical activity, and feed/water intake. The study found that LPS-challenged pigs retained 15% less of the available metabolizable energy and showed 25% less nutrient deposition. These results show concrete metabolic consequences caused by the febrile response to endotoxemia we discussed above.

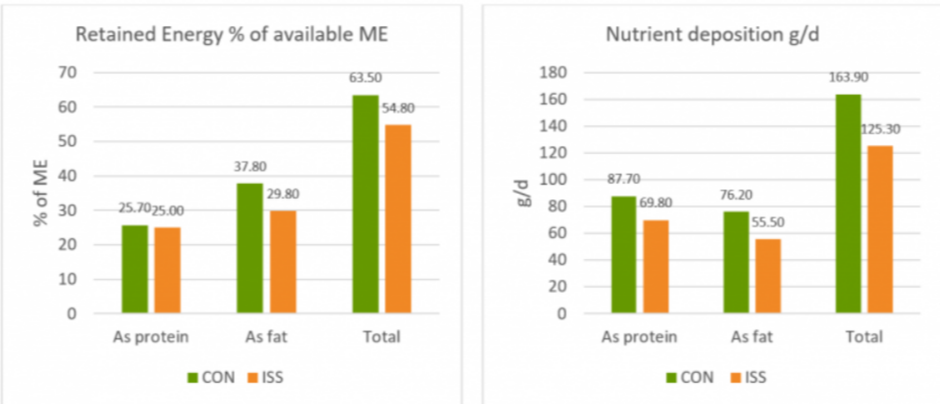


Figure 4: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from [Huntley, Nyachoti, and Patience, 2017](#))

Control treatment (CON) = Pigs fed by a basal diet
Immune system stimulation treatment (ISS) = Pigs given LPS (E. coli serotype 055:B5) injection

A loss of energy retained due to a reduction in available metabolizable energy leads to losses in performance as the amount of energy available for muscle production and fat storage will be lower. Furthermore, the decrease in feed intake creates a further energy deficit concerning production needs.

A [trial carried out at the University of Illinois](#) examined the effects of repeated injections of 400 µg *E. coli* LPS on chick performance from 11 to 22 days after hatching. The chicks were fed casein-based diets with graded levels of arginine. LPS administration reduced weight gain ($P<0.05$) and feed intake, and these effects tended to be worse at higher levels of arginine supplementation (Figure 5). The researchers hypothesize that, in response to endotoxin and elevated cytokine levels, macrophages use more arginine to produce nitric oxide, diverting it from protein production for muscle development.

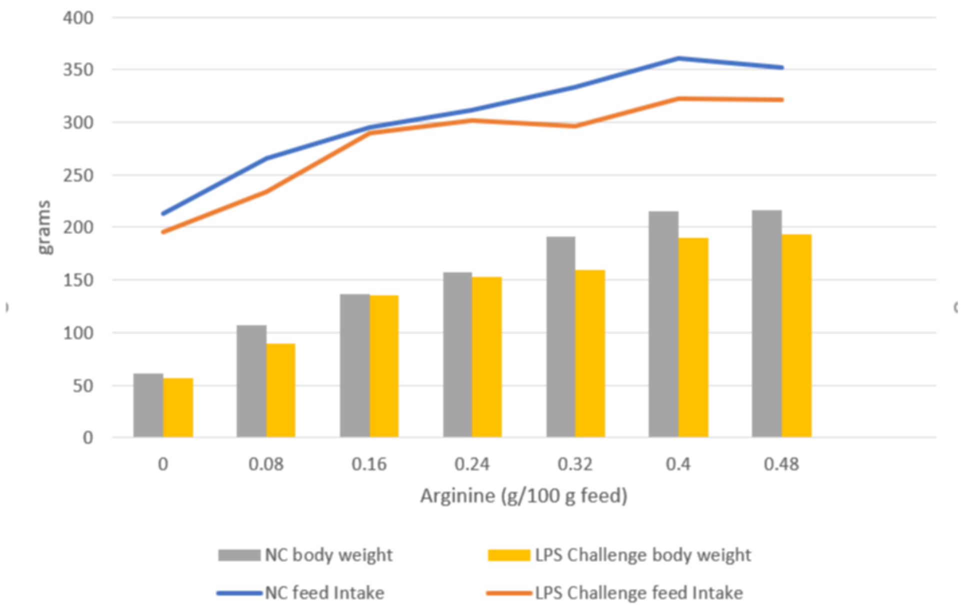


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on Webel, Johnson, and Baker, 1998)

NC = negative control

This data on poultry complements the results for swine, again showing that endotoxin-induced energy losses quantifiably depress animal performance even in milder disease cases.

The way forward: Endotoxin mitigation

Animals suffering from endotoxemia are subject to severe metabolic dysfunctions. If they do not perish from septic shock, they are still likely to show performance losses. Moreover, they at great risk of immunosuppression caused by the immune system "overdrive." Effective endotoxin mitigating agents can help to prevent these scenarios.

EW Nutrition's Mastersorb Gold is not only a leading anti-mycotoxin agent; thanks to its specific components, it effectively binds bacterial toxins. An *in vitro* study conducted at the Hogeschool Utrecht laboratory (part of Utrecht University) evaluated the binding capacity of Mastersorb Gold on LPS compared to three different competitor products. All products were tested at two different inclusion rates. At an inclusion rate of 0.25%, only Mastersorb Gold reduced the toxin load on the solution by 37%. At 1% inclusion, Mastersorb Gold bound 75% of the toxin, while only one competitor product demonstrated any binding (10%).

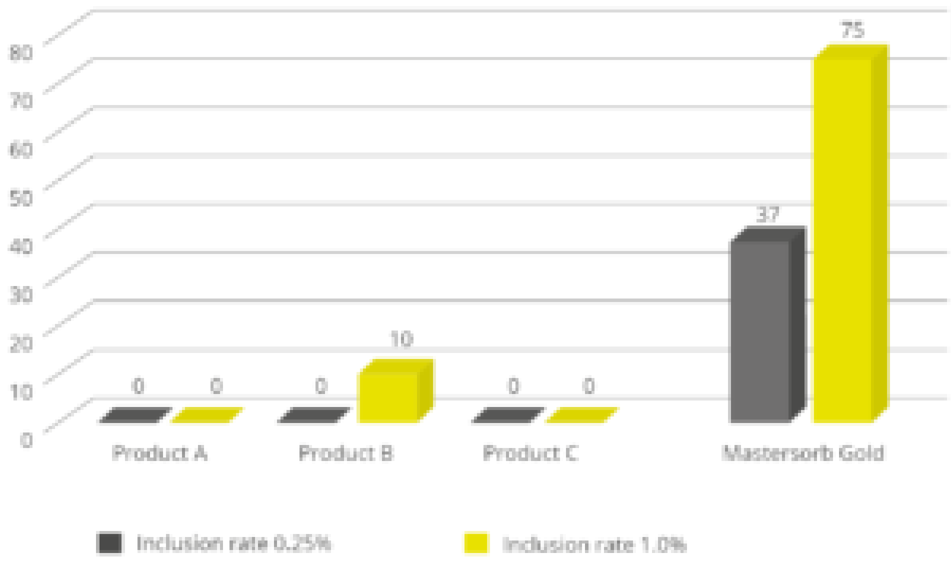


Figure 6: LPS adsorption capacity (%) – Mastersorb Gold clearly outperforms other anti-endotoxin products

Lipopolysaccharides are a constant challenge for animal production. The quantity of Gram-negative bacteria in an animal intestine is considerable; therefore, the danger of immune system over-stimulation through endotoxins cannot be taken lightly. Producers need to prioritize the maintenance of intestinal eubiosis in production animals proactively; for instance, through targeted gut health-enhancing additives based on phytomolecules and, possibly, organic acids.

Most importantly, the detrimental impact of LPS can be mitigated by using a high-performance agent such as Mastersorb Gold. To limit losses from an energy point of view yields positive results in terms of production levels and the prevention of secondary infections, preserving animal health and farms' economic viability.

References

Adib-Conquy, Minou, and Jean-Marc Cavaillon. "Compensatory Anti-Inflammatory Response Syndrome." *Thrombosis and Haemostasis* 101, no. 01 (2009): 36–47. https://doi.org/10.1160/th08-07-0421.

Huntley, Nichole F., C. Martin Nyachoti, and John F. Patience. "Immune System Stimulation Increases Nursery Pig Maintenance Energy Requirements." *Iowa State University Animal Industry Report* 14, no. 1 (2017). https://doi.org/10.31274/ans_air-180814-344.

Li, Jiaolong, Yongqing Hou, Dan Yi, Jun Zhang, Lei Wang, Hongyi Qiu, Binying Ding, and Joshua Gong. "Effects of Tributyrin on Intestinal Energy Status, Antioxidative Capacity and Immune Response to Lipopolysaccharide Challenge in Broilers." *Asian-Australasian Journal of Animal Sciences* 28, no. 12 (2015): 1784–93. https://doi.org/10.5713/ajas.15.0286.

Mani, Venkatesh, James H Hollis, and Nicholas K Gabler. "Dietary Oil Composition Differentially Modulates Intestinal Endotoxin Transport and Postprandial Endotoxemia." *Nutrition & Metabolism* 10, no. 1 (2013): 6. https://doi.org/10.1186/1743-7075-10-6.

Webel, D.M., R.W. Johnson, and D.H. Baker. "Lipopolysaccharide-Induced Reductions in Body Weight Gain and Feed Intake Do Not Reduce the Efficiency of Arginine Utilization for Whole-Body Protein Accretion in the Chick." *Poultry Science* 77, no. 12 (1998): 1893–98. https://doi.org/10.1093/ps/77.12.1893.

Zachary, James F. "Chapter 4 – Mechanisms of Microbial Infections." Essay. In *Pathologic Basis of Veterinary Disease*, 132–241. St Louis, MO: Mosby, 2017. https://doi.org/10.1016/B978-0-323-35775-3.00004-7.

Phytomolecules: Boosting Poultry Performance without Antibiotics

Antimicrobial resistance (AMR) is a major threat to global public health. It is largely caused by the overuse of antibiotics in human medicine and agriculture. In intensive poultry production most antibiotics are used as antimicrobial growth promoters and/or used as prophylactic and metaphylactic treatments to healthy animals. Reducing such antibiotic interventions is crucial to lowering the incidence of AMR. However, antibiotic reduction often results in undesirable performance losses. Hence alternative solutions are needed to boost poultry performance. Phytomolecules have antimicrobial, digestive, anti-inflammatory and antioxidant properties, which could make them key to closing the performance gap.

Poultry performance depends on intestinal health

Poultry performance is to a large extent a function of intestinal health. The intestines process nutrients, electrolytes and water, produce mucin, secrete immunoglobulins and create a barrier against antigens and pathogens.

In addition, it is an important component of the body's immune defense system. The intestine has to identify pathogens and reject them, but also has to tolerate harmless and beneficial microorganisms. If the intestines do not function properly this can lead to food intolerance, dysbiosis, infections and diseases. All of these are detrimental to feed conversion and therefore also to animal performance.

Antibiotics reduce the number of microorganisms in the intestinal tract. From a performance point of view this has two benefits: first, the number of pathogens is reduced and therefore also the likelihood of diseases; second, bacteria are eliminated as competitors for the available nutrients. However, the overuse of antibiotics not only engenders AMR: antibiotics also eliminate probiotic bacteria, which negatively impacts the digestive tracts' microflora.

Products to boost poultry performance may be added to their feed or water. They range from pre- and probiotics to medium chain fatty acids and organic acids to plant extracts or phytomolecules. Especially the latter have the potential to substantially reduce the use of antibiotics in poultry farming.

Phytomolecules are promising tools for antibiotic reduction

Plants produce phytomolecules to fend off pathogens such as moulds, yeasts and bacteria. Their antimicrobial effect is achieved through a variety of complex mechanisms. Terpenoids and phenols, for example, disturb or destroy the pathogens' cell wall. Other phytomolecules inhibit their growth by influencing their genetic material. Studies on broilers show that certain phytomolecules reduce the adhesion of pathogens such as to the wall of the intestine. Carvacrol and thymol were found to be effective against different species of *Salmonella* and *Clostridium perfringens*.

There is even evidence that secondary plant compounds also possess antimicrobial characteristics against antibiotic resistant pathogens. In-vitro trials with cinnamon oil, for example, showed antimicrobial effects against methicillin resistant Staphylococcus aureus, as well as against multiresistant E. coli, Klebsiella pneumoniae and Candida albicans.

Importantly, there are no known cases to date of bacteria developing resistances to phytomolecules. Moreover, phytomolecules increase the production and activity of digestive enzymes, they suppress the metabolism of pro-inflammatory prostaglandins and they act as antioxidants. Their properties thus make them a promising alternative to the non-therapeutic use of antibiotics.

Study design and results

In order to evaluate the effect of phytomolecules on poultry performance, multiple feeding studies were conducted on broilers and laying hens. They were given a phytogenic premix (Activo, EW Nutrition GmbH) that contains standardized amounts of selected phytomolecules.

To achieve thermal stability during the feed processing and a targeted release in the birds' gastrointestinal tract, the product is microencapsulated. For each , the studies evaluated both the tolerance of the premix and the efficacy of different dosages.

Study I: Evaluation of the dose dependent efficacy and tolerance of Activo for broilers
Animals: 400 broilers; age: 1-35 days of age
Feed: Basal starter and grower diets
Treatments:
– No supplement (negative control)
– 100 mg of Activo /kg of feed
– 1.000 mg of Activo /kg of feed
– 10.000 mg of Activo /kg of feed
Parameters: weight gain, feed intake, feed conversion ratio, health status, and blood parameters

Results: The trial group given the diet supplemented with 100 mg/kg Activo showed significant improvements in body weight gain during the starter period (+4%) compared to the control group. Additional significant improvements in feed conversion ratio (FCR) in the growing period (+4%) resulted in an overall improvement in FCR of 3%. At a 1.000 mg/kg supplementation, a significant improvement in FCR of 6% was observed over the entire feeding period. Hematological parameters were within the reference range of healthy birds when feeding up to 10,000 Activo/ kg of feed.

Study II: Evaluation of the dose depending efficacy and tolerance of Activo for laying hens

Animals: 200 hens; age: 20 to 43 weeks
Feed: basal diet for laying hens
Treatments:
– No supplement (negative control)
– 100 mg of Activo/ kg of feed
– 250 mg of Activo/ kg of feed
– 500 mg of Activo/ kg of feed
– 5.000 mg of Activo/ kg of feed
Parameters: weight gain, feed intake, feed conversion ratio, health status, and blood parameters

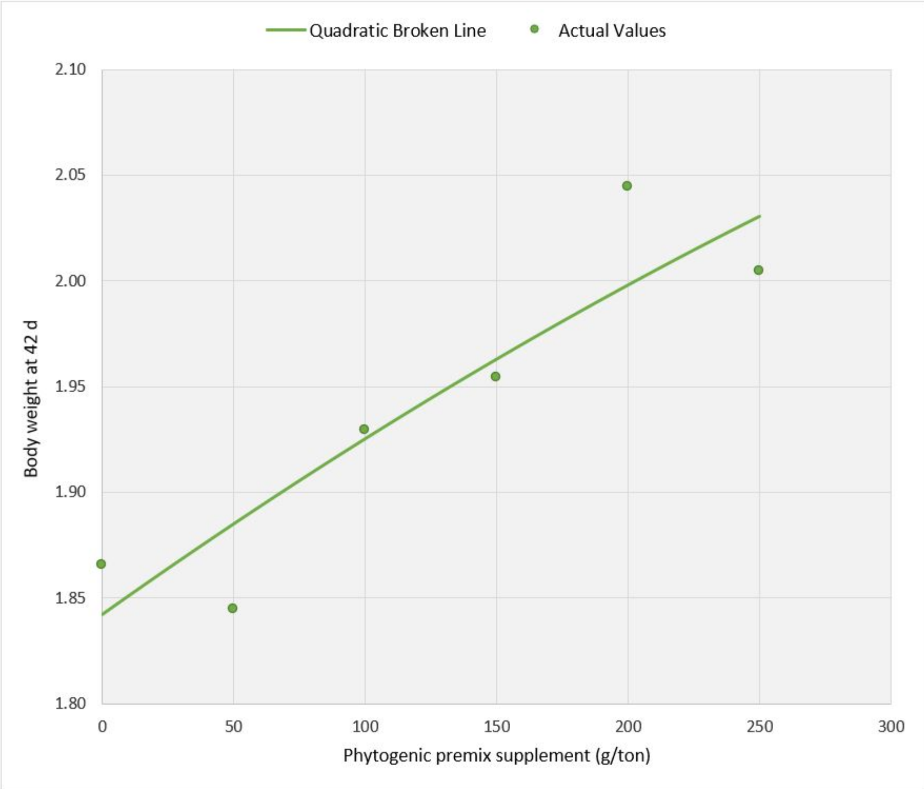
Results: Inclusion levels from 100 mg/kg of Activo onwards improved laying performance, egg mass and egg weight and reduced FCR compared to the control group. Results recorded for hematological parameters were within the reference range of healthy birds when feeding up to 5.000 mg Activo/ kg of feed.

Study III: Evaluation of the dose-dependent effects of Activo for coccidiosis vaccinated broilers

Animals: 960 broiler chickens; age: 42 days
Feed: Standard starter and finisher feed
Treatments:
– No supplement (negative control)
– 50 g of Activo /US ton of feed
– 100 g of Activo /US ton of feed
– 150 g of Activo /US ton of feed
– 200 g of Activo /US ton of feed
– 250 g of Activo /US ton of feed
– Antibiotic growth promoter (AGP)(positive control)
Parameters: weight gain, feed efficiency
Specific: In order to represent field conditions, the birds were challenged with used, homogenized litter.

Results: A clear dose response for both body weight gain and feed efficiency was observed (see Figure 1): the more phytogenic premix given, the better the birds' performance. The group with 200g of Activo /US ton of feed showed similar performance levels than the positive control group supplemented with AGP.

Figure 1: Dose-dependent effects of for coccidiosis vaccinated broilers



Study IV: Evaluation of the dose-dependent effects of Activo for laying hens

Animals: 40 hens; age: week 20 to 43
Feed: basal diet for laying hens
Treatments:
– No supplement (negative control)
– 100 mg of Activo/ kg of feed
– 250 mg of Activo/ kg of feed
– 500 mg of Activo/ kg of feed
– 5.000 mg of Activo/ kg of feed
Parameters: weight gain, feed intake, egg production, feed conversion ratio, health status
Duration: 168 days of feeding period

Results: The laying hens showed a higher laying rate when fed with a higher concentration of phytomolecules (Figure 2). Similarly improved results were observed for the feed efficiency. The more phytogenic premix added to their diet the better feed efficiency (Figure 3).

Figure 2: Dose-dependent effects of Activo on laying rate in laying hens

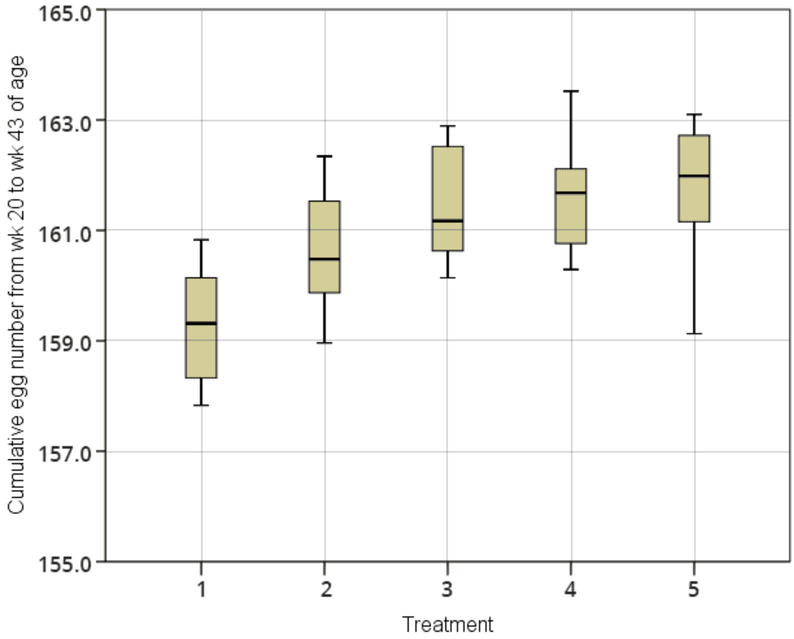
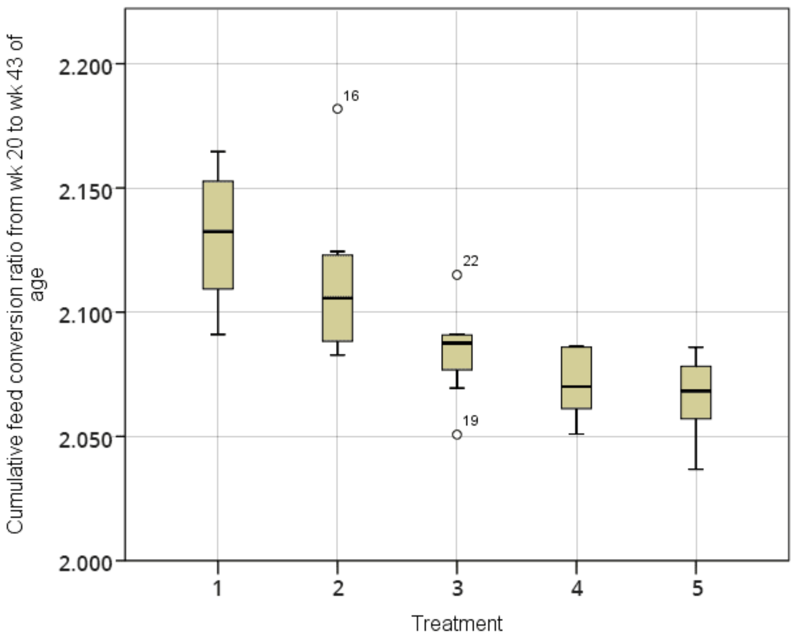


Figure 3: Dose-dependent effects of Activo on feed efficiency in laying hens



In conclusion, all four studies indicate that the inclusion of phytomolecules in broilers' and laying hens' diet improves their performance. Increasing levels of a phytogenic premix (Activo) significantly increased the production parameters for both groups. These improvements might bring performance in antibiotic-free poultry production on par with previous performance figures achieved with antimicrobial growth promoters.

The studies also showed that microencapsulated phytogenic premixes are safe when used in dose ranges recommended by the suppliers. No negative effects on animal health could be observed even at a 100 fold / 50 fold of the recommended inclusion rate in diets for broiler or laying hens, respectively. Thanks to their positive influence on intestinal health, phytomolecules thus boost poultry performance in a safe and effective way.

By Technical Team, EW Nutrition

Literature

Alanis, Alfonso J. "Resistance to Antibiotics: Are We in the Post-Antibiotic Era?" Archives of Medical Research 36, no. 6 (October 08, 2005): 697-705. doi:10.1016/j.arcmed.2005.06.009.

Borda-Molina, Daniel, Jana Seifert, and Amélia Camarinha-Silva. "Current Perspectives of the Chicken Gastrointestinal Tract and Its Microbiome." Computational and Structural Biotechnology Journal 16 (March 15, 2018): 131-39. doi:10.1016/j.csbj.2018.03.002.

Diaz-Sanchez, Sandra, Doris Dsouza, Debrabrata Biswas, and Irene Hanning. "Botanical Alternatives to Antibiotics for Use in Organic Poultry Production." Poultry Science 94, no. 6 (June 2015): 1419-430. doi:10.3382/ps/pev014.

Du, Encun, Weiwei Wang, Liping Gan, Zhui Li, Shuangshuang Guo, and Yuming Guo. "Effects of Thymol and Carvacrol Supplementation on Intestinal Integrity and Immune Responses of Broiler Chickens Challenged with Clostridium Perfringens." Journal of Animal Science and Biotechnology 7, no. 19 (March 22, 2016). doi:10.1186/s40104-016-0079-7.

Gao, Pengfei, Chen Ma, Zheng Sun, Lifeng Wang, Shi Huang, Xiaoquan Su, Jian Xu, and Heping Zhang. "Feed-additive Probiotics Accelerate Yet Antibiotics Delay Intestinal Microbiota Maturation in Broiler Chicken." Microbiome 5, no. 1 (August 03, 2017). doi:10.1186/s40168-017-0315-1.

Khan, Rosina, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad Ahmad, S. Manazir Ali, Mashiatullah Siddiqui, and Asad Khan. "Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin." Molecules 14, no. 2 (February 04, 2009): 586-97. doi:10.3390/molecules14020586.

Manafi, Milad, Mahdi Hedayati, Saeed Khalaji, and Mohammad Kamely. "Assessment of a Natural, Non-antibiotic Blend on Performance, Blood Biochemistry, Intestinal Microflora, and Morphology of Broilers Challenged with Escherichia Coli." Revista Brasileira De Zootecnia 45, no. 12 (December 2016): 745-54. doi:10.1590/s1806-92902016001200003.

Photo source: Aviagen