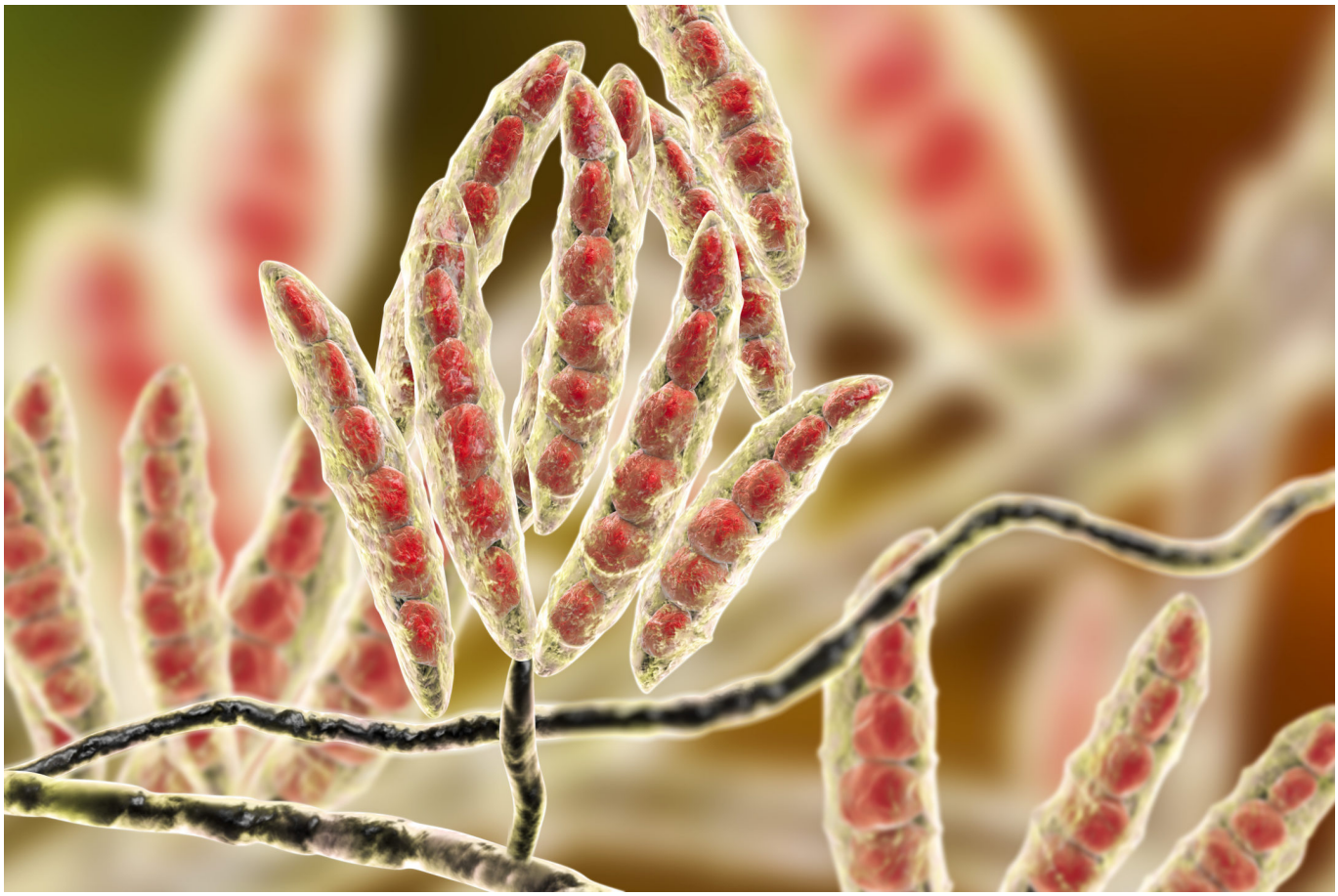
A complex battlefield: mycotoxins in the gastrointestinal tract



Most grains used as feed raw materials are susceptible to mycotoxin contamination. These toxic secondary metabolites are produced by fungi before or after harvest and cause severe economic losses all along agricultural value chains. For livestock, negative consequences include acute effects such as impaired liver and kidney function, vomiting, or anorexia, as well as chronic effects such as immunosuppression, growth retardation, and reproductive problems. Mycotoxin management is, therefore, of the utmost priority for animal producers worldwide.

But how is it that mycotoxins cause such damage in the first place? This article delves into the complex processes that take place when mycotoxins come into contact with the gastrointestinal tract (GIT). The intestinal epithelium is the first tissue to be exposed to mycotoxins, and often at higher concentrations than other tissues. A deeper understanding of how mycotoxins affect the GIT allows us to appreciate the cascading effects on animal health and performance, why such damage already occurs at contamination levels well below official safety thresholds – and what we can do about it.

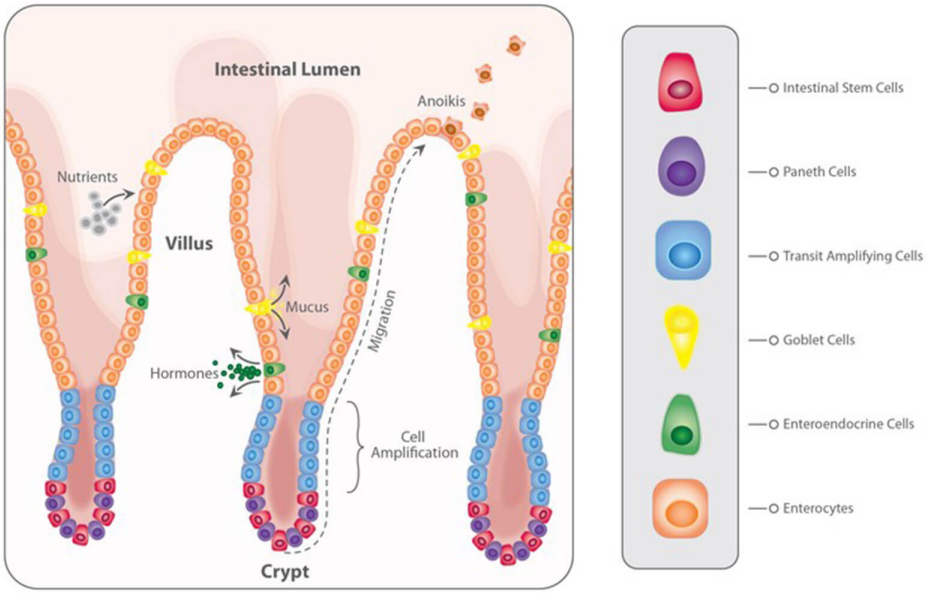
The intestinal epithelium: the busy triage site for nutrients and harmful substances

When mycotoxins are ingested, they encounter the GIT's intestinal epithelium (Figure 1). This single layer of cells lining the intestinal lumen serves two conflicting functions: firstly, it must be permeable enough to allow the absorption of nutrients. On the other hand, it constitutes the primary physiological barrier against harmful agents such as viruses, microorganisms, and toxins.

Within the intestinal epithelium, several types of highly specialized cells are involved in epithelial regeneration, nutrient absorption, innate defense, transport of immunoglobulins, and immune surveillance. The selective barrier function is maintained due to the formation of complex networks of proteins that link adjacent cells and seal the intercellular space. Besides, the intestinal epithelium is covered with mucus produced by goblet cells, which isolates its surface, preventing the adhesion of pathogens to the enterocytes (intestinal absorptive cells).

Due to its dual involvement in digestive and immune processes, the intestinal epithelium plays a pivotal role in the animal's overall health. Importantly, the epithelium is directly exposed to the entire load of ingested mycotoxins. Hence their effects can be problematic even at low concentrations.

Figure 1: The intestinal epithelium



Problematic effects of mycotoxins on the intestinal epithelium

Most mycotoxins are absorbed in the proximal part of the gastrointestinal tract (Table 1). This absorption can be high, as in the case of aflatoxins (~90%), but also very limited, as in the case of fumonisins (<1%); moreover, it depends on the species. Importantly, a significant portion of unabsorbed toxins remains within the lumen of the gastrointestinal tract.

Some of the mycotoxins that enter the intestinal lumen can be bio-transformed into less toxic compounds by the action of certain bacteria. This action, however, predominantly happens in the large intestine – therefore, no detoxification takes place before absorption in the upper parts of the GIT. Part of the absorbed mycotoxins can also re-enter the intestine, reaching the cells from the basolateral side via the bloodstream. Furthermore, they re-enter through enterohepatic circulation (the circulation of substances between the liver and small intestine). Both actions increase the gastrointestinal tract's overall exposure to the toxins.

Table 1: Rate and absorption sites of different mycotoxins

Mycotoxin	Primary absorption sites	Absorption rate in poultry	Absorption rate in swine	Observations
Aflatoxin	Duodenum / Jejunum	~90%	~80%	Subject to enterohepatic circulation
DON	Duodenum / Jejunum	Up to 20%	Up to 60%	Subject to enterohepatic circulation and to biotransformation in the large intestine
Fumonisin	Duodenum / Jejunum	Less than 1%	Less than 5%	Subject to enterohepatic circulation
Ochratoxin	Jejunum	~40%	~60%	Subject to enterohepatic circulation and to biotransformation in the large intestine
Zearalenone	Small & large intestine	~10%	~80%	Subject to enterohepatic circulation

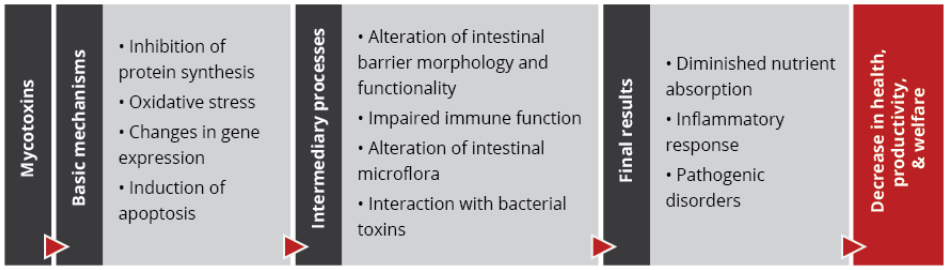
Adapted from: Biehl et al., 1993; Bouhet & Oswald, 2007; Devreese et al., 2015; Ringot et al., 2006

The damaging impact of mycotoxins on the intestinal epithelium initially occurs through:

- A decrease in protein synthesis, which reduces barrier and immune function (Van de Walle et al., 2010)
- Increased oxidative stress at the cellular level, which leads to lipid peroxidation, affecting cell membranes (Da Silva et al., 2018)
- Changes in gene expression and the production of chemical messengers (cytokines), with effects on the immune system and cellular growth and differentiation (Ghareeb et al., 2015)
- The induction of programmed cell death (apoptosis), affecting the reposition of immune and absorptive cells (Obremski & Poniatowska-Broniek, 2015)

Importantly, studies based on realistic mycotoxin challenges (e.g., Burel et al., 2013) show that the mycotoxin levels necessary to trigger these processes are lower than the levels reported as safe by EFSA, the Food Safety Agency of the European Union. The ultimate consequences range from diminished nutrient absorption to inflammatory responses and pathogenic disorders in the animal (Figure 2).

Figure 2: Mycotoxins' impact on the GIT and consequences for monogastric animals



1. Alteration of the intestinal barrier's morphology and functionality

The mycotoxins DON, fumonisin, and T2 induce a reduction in the rate of epithelial cell proliferation and differentiation. This causes a decrease in the height and the surface of the intestinal villi, which in turn leads to a reduction in nutrient absorption. Additionally, some nutrient transporters are inhibited by the action of mycotoxins such as DON and T2, for example, negatively affecting the transport of glucose.

Several studies indicate that mycotoxins such as aflatoxin B1, DON, fumonisin B1, ochratoxin A, and T2, can increase the permeability of the intestinal epithelium of poultry and swine (e.g. Pinton & Oswald, 2014). This is mostly a consequence of the inhibition of protein synthesis. As a result, there is an increase

in the passage of antigens into the bloodstream (e.g., bacteria, viruses, and toxins). This increases the animal's susceptibility to infectious enteric diseases. Moreover, the damage that mycotoxins cause to the intestinal barrier entails that they are also being absorbed at a higher rate.

2. Impaired immune function in the intestine

The intestine is a very active immune site, where several immuno-regulatory mechanisms simultaneously defend the body from harmful agents. Immune cells are affected by mycotoxins through the initiation of apoptosis, the inhibition or stimulation of cytokines, and the induction of oxidative stress. Studies demonstrate that aflatoxin, DON, fumonisin, T2, and zearalenone interact with the intestinal immune system in such a manner that the animal's susceptibility to viral and bacterial infections increases (e.g., Burel et al., 2013). Moreover, by increasing their fecal elimination, the horizontal transmission of pathogens is extended.

For poultry production, one of the most severe enteric problems of bacterial origin is necrotic enteritis, which is caused by *Clostridium perfringens* toxins. Any agent capable of disrupting the gastrointestinal epithelium – e.g. mycotoxins such as DON, T2, and ochratoxin – promotes the development of necrotic enteritis. The inhibition of the intestinal immune system caused by mycotoxins such as aflatoxin, DON, and T2 also collaborates with the development of this disease.

3. Alteration of the intestinal microflora

The gastrointestinal tract is home to a diverse community of bacteria, fungi, protozoa, and viruses, which lines the walls of the distal part of the intestine. This microbiota prevents the growth of pathogenic bacteria through competitive exclusion and the secretion of natural antimicrobial compounds, volatile fatty acids, and organic acids.

Recent studies on the effect of various mycotoxins on the intestinal microbiota show that DON and other trichothecenes favor the colonization of coliform bacteria in pigs. DON and ochratoxin A also induce a greater invasion of *Salmonella* and their translocation to the bloodstream and vital organs in birds and pigs – even at non-cytotoxic concentrations. It is known that fumonisin B1 may induce changes in the balance of sphingolipids at the cellular level, including for gastrointestinal cells. This facilitates the adhesion of pathogenic bacteria, increases in their populations, and prolongs infections, as has been shown for the case of *E. coli*.

From the perspective of human health, the colonization of the intestine of food-producing animals by pathogenic strains of *E. coli* and *Salmonella* is of particular concern. Mycotoxin exposure may well increase the transmission of these pathogens, posing a risk for human health.

4. Interaction with bacterial toxins

When mycotoxins induce changes in the intestinal microbiota, this can lead to an increase in the endotoxin concentration in the intestinal lumen. Endotoxins or lipopolysaccharides (LPS) are fragments of Gram-negative bacteria's cell walls. They are released during bacterial cell death, growth, and division. Hence endotoxins are always present in the intestine, even in healthy animals. Endotoxins promote the release of several cytokines that induce an enhanced immune response, causing inflammation, thus reducing feed consumption and animal performance, damage to vital organs, sepsis, and death of the animals in some cases.

The synergy between mycotoxins and endotoxins can result in an overstimulation of the immune system. The interaction between endotoxins and estrogenic agents such as zearalenone, for example, generates chronic inflammation and autoimmune disorders because immune cells have estrogen receptors, which are stimulated by the mycotoxin. The combination of DON at low concentrations and endotoxins in the intestine, on the other hand, has been shown to engender a decrease in transepithelial resistance and to alter the balance of the microbiota.

What to do? Proactive toxin risk management

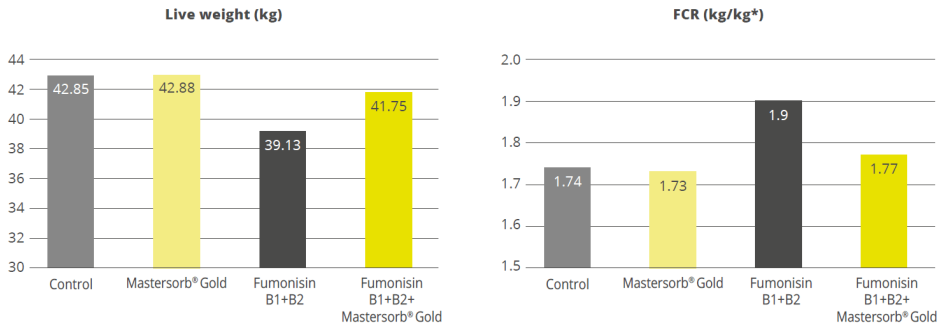
To prevent the detrimental consequences of mycotoxins on animal health and performance, proactive solutions are needed that support the intestinal epithelium's digestive and immune functionality and help maintain a balanced microbiome in the GIT. Moreover, it is crucial for any anti-mycotoxin product to feature both anti-mycotoxin and anti-bacterial toxin properties and that it supports the organs most targeted by mycotoxins, e.g., the liver. EW Nutrition's Mastersorb Gold premix is based on the synergistic combination of natural clay minerals, yeast cell walls, and phytomolecules. Its efficacy has been extensively tested, including as a means for dealing with *E. coli* endotoxins.

Mastersorb Gold: anti-mycotoxin activity stabilizes performance and strengthens liver health

A field trial conducted in Germany on male Ross 308 broilers showed that for broilers receiving a diet contaminated with DON and zearalenone, adding 1kg of Mastersorb Gold per ton of feed to their diet led to significant performance enhancements. Not only did they recuperate the mycotoxin-induced weight loss (6% increase relative to the group receiving only the challenge), but they gained weight relative to the control group (which received neither the challenge nor Mastersorb Gold). Feed conversion also improved by 3% relative to the group challenged with mycotoxins.

A scientific study of crossbred female pigs showed that Mastersorb Gold significantly reduced the deleterious effects of fumonisin contamination in the feed. The decrease in weight gain and the decline of feed conversion could be mitigated by 6.7% and 13 FCR points, respectively (Figure 3). Also, the sphinganine/sphingosine (Sa/So) ratio, a biomarker for fumonisin presence in the blood serum, could be decreased by 22.5%.

Figure 3: Mastersorb Gold boosts performance for pigs fed a fumonisin-contaminated diet

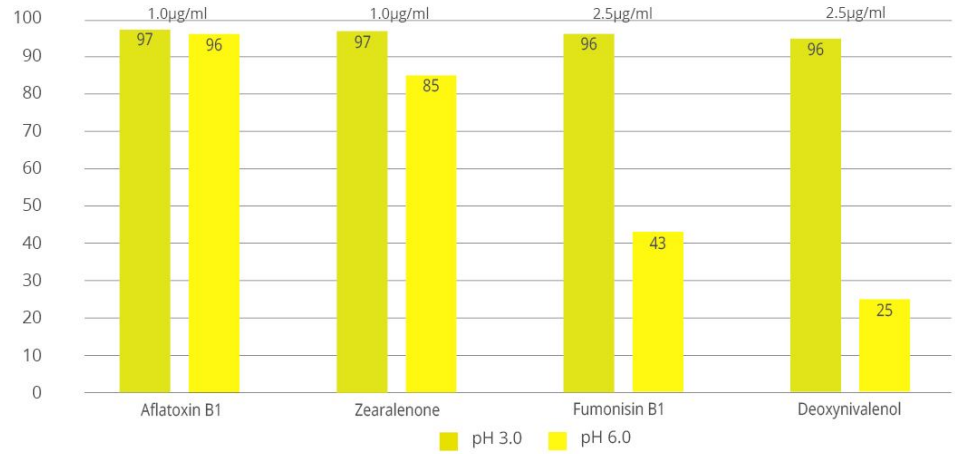


Another study of crossbred female piglets, carried out at a German university, investigated whether Mastersorb Gold could support performance as well as liver health under a naturally occurring challenge of ZEA (~ 370ppb) and DON (~ 5000ppb). Mastersorb Gold significantly improved weight gain and feed conversion in piglets receiving the mycotoxin-contaminated diet: daily body weight gain was 75g higher than that of a group receiving only the challenge, and the FCR improved by 24% (1.7 vs. 2.25 for the group without Mastersorb Gold). Moreover, Mastersorb Gold significantly improved the liver weight (total and relative) and the piglets' AST levels (aspartate aminotransferase, an enzyme indicating liver damage). A tendency to improve spleen weight and GGT levels (gamma-glutamyl transferase, another enzyme indicative of liver issues) was also evident, all of which indicate that Mastersorb Gold effectively counteracts the harmful impact of mycotoxin contamination on liver functionality.

In-vitro studies demonstrate Mastersorb Gold's effectiveness against mycotoxins as well as bacterial toxins

Animal feed is often contaminated with two or more mycotoxins, making it important for an anti-mycotoxin agent to be effective against a wide range of different mycotoxins. Besides, to prevent mycotoxins damaging the GIT, an effective product should ideally adsorb most mycotoxins in the first part of the animal's intestine (under acidic conditions). In-vitro experiments at an independent research facility in Brazil showed that an application of 0.2% Mastersorb Gold binds all tested mycotoxins at rates from 95 to 97% at a pH level of 3, using realistic challenges of 1000ppb (Aflatoxin B1 and ZEA) and 2500ppb (Fumonisin B1 and DON). The binding results achieved for Fumonisin and DON, which are often considered outright "nonbinding," under challenging close to neutral conditions (pH 6), are particularly encouraging.

Figure 4: Mastersorb Gold binding capacity against different mycotoxins (%)



Concerning its efficacy against endotoxins, an *in vitro* study conducted at Utrecht University, among other studies, has shown Mastersorb Gold to be a strong tool against the LPS released by *E. coli.* For the test, four premium mycotoxin binders were suspended in a phosphate buffer solution to concentrations of 0.25% and 1%. *E. coli* LPS were suspended to a final concentration in each sample of 50ng/ml. Against this particularly high challenge, Mastersorb Gold achieved a binding rate of 75% at an inclusion rate of 1%: clearly outperforming competing products, which at best showed a binding rate of 10%.

Conclusion

A healthy gastrointestinal tract is crucial to animals' overall health: it ensures that nutrients are optimally absorbed, it provides effective protection against pathogens through its immune function, and it is key to maintaining a well-balanced microflora. Even at levels considered safe by the European Union, mycotoxins can compromise different intestinal functions such as absorption, permeability, immunity, and microbiota balance, resulting in lower productivity and susceptibility to disease.

To safeguard animal performance, it is important to continually strive for low levels of contamination in feed raw materials – and to stop the unavoidable mycotoxin loads from damaging the intestinal epithelium through the use of an effective anti-mycotoxin agent, which also supports animals against endotoxins and boosts liver function. Research shows that Mastersorb Gold is a powerful tool for proactive producers seeking stronger animal health, welfare, and productivity.

By Marisabel Caballero, Global Technical Manager Poultry, EW Nutrition

Poultry health and welfare: Phytomolecules for poultry diets

The large majority of poultry specialists in Europe consider phytomolecules as one of the key elements in diets for broilers, broiler breeders, and layers when birds are raised without antibiotics. A quick glance at the market will reveal more commercial products than can possibly be imagined. There are three basic elements you should bear in mind when making your choice:

1. **Most phytomolecules are volatile**. As such, unprotected products will soon evaporate if left exposed to the open air – as it happens, for instance, with feed prepared in commercial farms. Microencapsulation is therefore essential.
2. **There are countless phytomolecules.** Consequently, finding the right mix for the task required is essential, as not all mixtures will get you the desired result. When designing a phytomolecule mix, the manufacturer must have the necessary knowledge and experience to achieve the desired result.
3. **Phytomolecules are powerful.** This is to say that you cannot just keep adding higher quantities to achieve a better result. Finding the exact inclusion rates for the right purpose is a difficult balancing exercise.

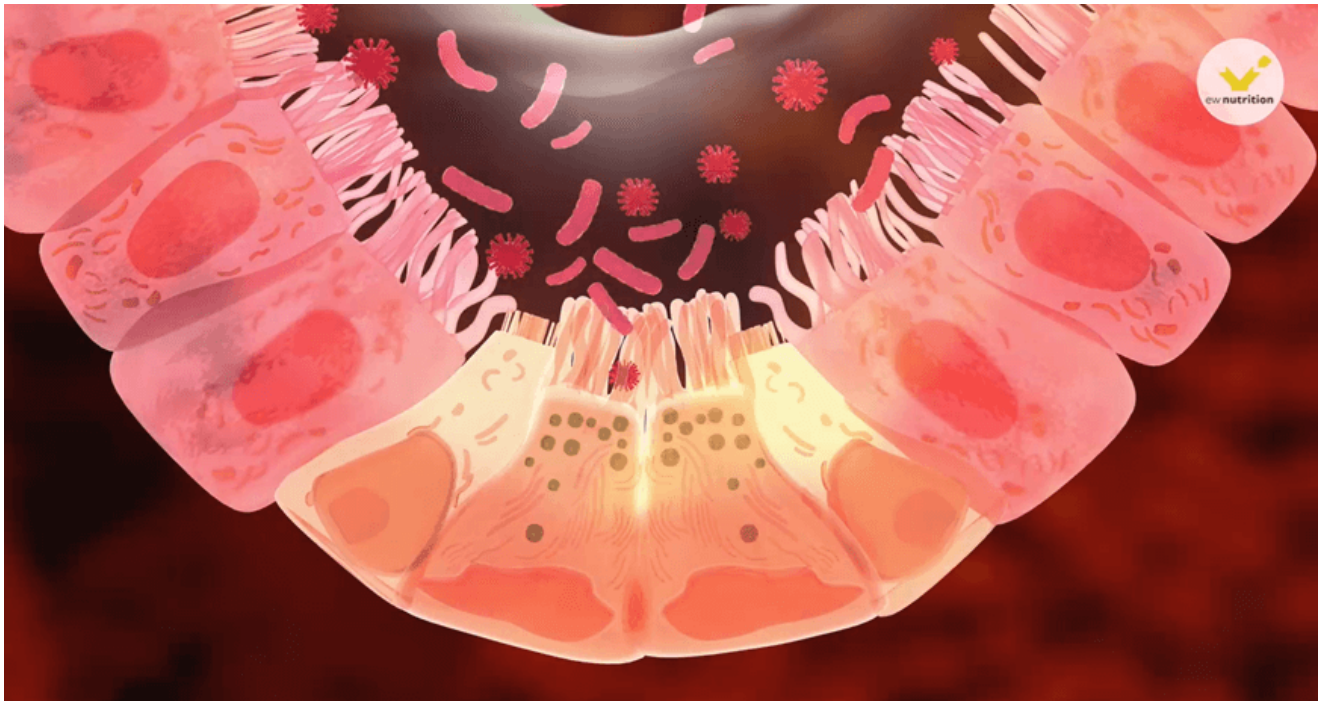
In fact, the right protection, the right mix and the right inclusion rates must be combined to ensure that

the animals do not refuse the feed (worst case scenario) or just fail to benefit from the inclusion of phytomolecules.

Among the feed additives, phytomolecules (or secondary plant compounds) stand out as a class of active ingredients that may help to improve gut health and thereby reduce the use of antibiotics. Synthesized by plants as a defense mechanism against pathogens, phytomolecules promote the digestion of feed ingredients (Zhai et al. 2018), prevent loss of gut integrity during enteric challenges (Liu et al. 2018), and have antimicrobial properties that hinder the growth of potential pathogens (Chowdhury, 2018). Phytomolecules can prevent the overgrowth of opportunistic pathogens, thereby reducing the frequency of occurrence of diseases such as necrotic enteritis and dysbacteriosis and thus improve performance data such as daily weight gain and feed efficiency.

Beyond the phytomolecules' proven effects, what works best in supporting the health and welfare of your animals is, in fact, a holistic program (such as those offered by EW Nutrition) that consists of an effective combination of innovative products and consultancy services in the fields of gut health, nutrition, AMR monitoring, and biosecurity management.

**This article is available in Dutch.*



Challenging times for broilers? Phytomolecules, not antibiotics, are the answer

*by **Ajay Bhoyar**, Global Technical Manager, EW Nutrition*

Anyone working with today's fast-growing broiler chicken knows that it is a sensitive creature – and so is its gut health. Thanks to continuous improvements in terms of genetics and breeding, nutrition and feeding, as well as general management strategies, broiler production has tremendously upped performance and efficiency over the past decades. It is estimated that, between 1957 and 2005, the broiler growth rate increased by over 400%, while the feed conversion ratio dropped by 50%.

These impressive improvements, however, have come at the cost of intense pressure on the birds' digestive system, which needs to process large quantities of feed in little time. To achieve optimal growth, a broiler's gastrointestinal tract (GIT) needs to be in perfect health, all the time. Unsurprisingly, enteric diseases such as necrotic enteritis, which severely damages the intestinal mucosa, hamper the intestines' capacity to absorb nutrients and induce an inflammatory immune response.

The modern broiler's gut – a high-performing,

but sensitive system

However, in a system as high performing as the modern broiler's GIT, much less can lead to problems. From when they are day-old chicks up to slaughter, broilers go through several challenging phases during which they are more likely to show impaired gut functionality, e.g. after vaccinations or feed changes. Good management practices go a long way towards eliminating unnecessary stressors for the animals, but some challenging periods are unavoidable.

The transition from starter to grower diets is a classic situation when nutrients are very likely to not be well digested and build up in the gut, fueling the proliferation of harmful microbes. Immunosuppressive stress in combination with an immature intestinal microflora results in disturbances to the bacterial microbiota. At "best", this entails temporarily reduce nutrient absorption, in the worst case the birds will suffer serious intestinal diseases.

Phytomolecules – the intelligent alternative to antibiotics

To safeguard performance during stressful periods, poultry producers need to anticipate them and proactively provide effective gut health support. For many years, this support came in the form of antibiotic growth promoters (AGP): administered prophylactically, they were effective at keeping harmful enteric bacteria in check. However, due to grave concerns about the development of antimicrobial resistance, non-therapeutic antibiotics use has been banned in many countries. Alternatives need to focus on improving feed digestibility and strengthening gut health, attacking the root causes of why the intestinal microflora would become unbalanced in the first place.

Phytomolecules are secondary metabolites active in the defense mechanisms of plants. Studies have found that certain phytomolecules stimulate digestive enzyme activities and stabilize the gut microflora, "leading to improved feed utilization and less exposure to growth-depressing disorders associated with digestion and metabolism" (Zhai et al., 2018). With other trials showing positive effects on broilers' growth performance and feed conversion, the research indicates that phytomolecules might also specifically support chickens during challenging phases.

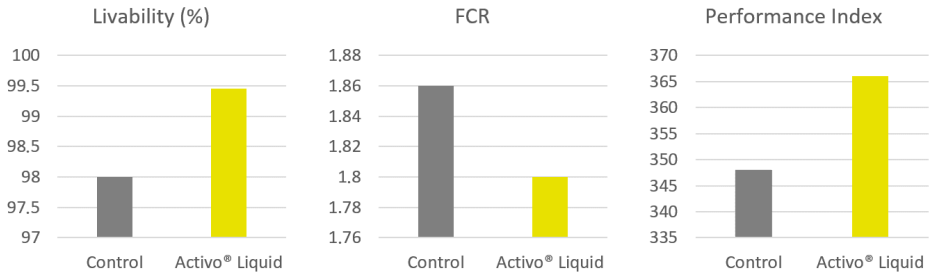
The effect of phytomolecules on broilers during a challenging phase

A study was conducted over a period of 49 days on a commercial broiler farm of an AGP-free integration operation in Japan. The farm reported gut health challenges in the second and third week of the fattening period due to vaccinations and changes to the animals' diets. The trial included 15504 Ross 308 broilers, divided into two groups. The negative control group included a total of 7242 birds, kept in another house.

All the birds were fed the standard feed of the farm. The trial group (8262 birds) received Activo Liquid, which contains a synergistic combination of phytomolecules, administered directly through the drinking water. Activo Liquid was given at an inclusion rate of 200ml per 1000L of water (3.3 US fl oz per gallon of stock solution, diluted at 1:128), from day 8 until day 25, for 8 hours a day.

The results are summarized in Figure 1:

Figure 1: Improved broiler performance for Activo Liquid group (day 49)



The Activo Liquid group clearly showed performance improvements compared to the control group. Livability augmented by 1.5%, while the feed conversion rate improved by 3.2%. This resulted in a more than 5% higher score in terms of the performance index.

Challenging times? Tackle them using phytomolecules

Poultry producers take great care to eliminate unnecessary sources of stress for their birds. Nonetheless, during their lifecycle, broiler chickens face challenging periods during which the balance of the intestinal microflora can easily become disturbed, with consequences ranging from decreased nutrient absorption to full-blown enteric disease.

The trial reviewed here showed that, after receiving Activo Liquid, broilers raised without AGPs showed encouraging performance improvements during a challenging phase of feed changes and vaccinations. Likely thanks to the activation of digestive enzymes and a stabilization of the gut flora, the broilers showed improved livability and feed conversion, thus delivering a much more robust performance during a critical phase of their lives. In times where the non-therapeutic use of antibiotics is no longer an option, phytomolecules allow poultry farmers to effectively support their animals during challenging times.

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Photo Source: Aviagen

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Want antibiotic-free broilers? Raise low-AB breeders

Strong demand by consumers; restaurant chains and wholesalers for antibiotic-free (ABF) meat; the threat of antimicrobial resistance; and stringent regulations on the use of antibiotics – there are many good reasons for poultry producers to strive for antibiotic-free production systems. Crucially, to successfully produce poultry meat without antibiotics requires a paradigm shift that starts right at the parent stock level, with the antibiotic-free production of hatching eggs.

Broiler breeders' gut health is linked to progeny's performance

Broiler breeders' performance is measured in terms of how many saleable day old chicks (DOCs) per hen they produce. However, within a sustainable ABF production system (also known as No Antibiotics Ever or NAE), this parameter is not seen in isolation. Breeder hens' nutritional and health status not only affect the number of DOCs they can produce, but also the transfer of nutrients, antibodies, microbiota and even contaminants, e.g. mycotoxins, to the egg – and therefore, their progeny's long-term health and performance.

This starts with egg formation, which requires several metabolic processes in the hen to function perfectly.

If the hen's intestinal integrity is compromised, for example due to mycotoxins, she will absorb fewer nutrients, which in turn affects egg formation. Mycotoxicosis has particularly insidious effects for egg formation as it can damage the liver whose biosynthetic activities strongly impact on the egg's internal (yolk) and external (eggshell) quality.

Chick embryos depend on the maternal antibodies and nutrients deposited in the yolk, including vitamin D3, carotenoids, and fatty acids, to develop normally. Eggshell quality, among other things, affects the embryo's access to oxygen, which is especially important when it develops body tissues.

Hens' ability to form healthy eggs depends on their diet and health. Research indicates that, via the impact on egg formation, broiler breeders' feeding program quantifiably influences their progeny's immune system and intestinal health. There is indeed a direct relationship between parent and offspring's gut health because the chick's microbiome is in part also inherited from the hen. The impact on DOC quality is thus one of many dimensions to consider when calibrating one's broiler breeders feeding approach.

The challenge of feeding an ABF broiler breeder

Just as their offspring, breeder hens are genetically predisposed for rapid growth and muscle development. From rearing right through to the laying period, poultry nutritionists need to carefully balance their diets and moderate weight gain in order for hens to reach their reproductive potential.

Different stages of a breeder's life cycle come with different objectives – for example, good flock uniformity in the rearing period versus egg size and hatchability in the laying phase – and thus different requirements in terms of calories, amino acids, vitamins, and minerals. What remains constant is that the actual nutrient intake depends on intestinal health, determining both the breeders' performance and, via the impact on egg characteristics, its progeny's performance.

The feeding regimes adopted to avoid hens becoming overweight can have a negative effect on their gut flora. Without antibiotics as a tool to maintain or recover optimal gut function, even mild intestinal disorders can quickly become chronical impairments that negatively impact breeders' productivity. In ABF production systems, intestinal health therefore needs to be a central focus for the feeding strategy.

Can phytomolecules improve broiler breeders' performance?

Among the plethora of feed additives, phytomolecules, or secondary plant compounds, stand out as a class of active ingredients that may help to improve gut health and thereby reduce the use of antibiotics. Synthesized by plants as a defense mechanism against pathogens, phytomolecules combine digestive, antimicrobial and antioxidant properties.

Some studies have shown that phytomolecules-based products can increase broilers' body weight gain and improve laying hens' laying rate, egg mass and egg weight. Both broilers and laying hens responded to the inclusion of phytomolecules in their diet with inclusion rate-dependent improvements in feed conversion. To evaluate if phytomolecules could similarly improve broiler breeders' performance, two trials were conducted.

Study I: Effect of phytomolecules on laying performance during peak production

The first study was set up on a farm in Thailand. In total, 40000 Cobb broiler breeders (85% female, 15% male) were divided into two groups with 8500 hens (one house) in the control and 25500 (three houses) in the trial group. Both groups were fed standard feed. The trial group additionally received a phytomolecules-based liquid complementary feed (Activo Liquid, EW Nutrition GmbH) via the waterline from week 24 to week 32 at a rate of 200ml/1000L during 5 days per week.

Activo Liquid was found to have a positive influence on laying performance (Figure 1). The average laying

rate increased by 7.2% during the trial period, resulting in almost 3 additional hatching eggs per hen housed. A further indication of the beneficial influence that this particular combination of phytomolecules had on gut health was a 0.2% lower mortality.

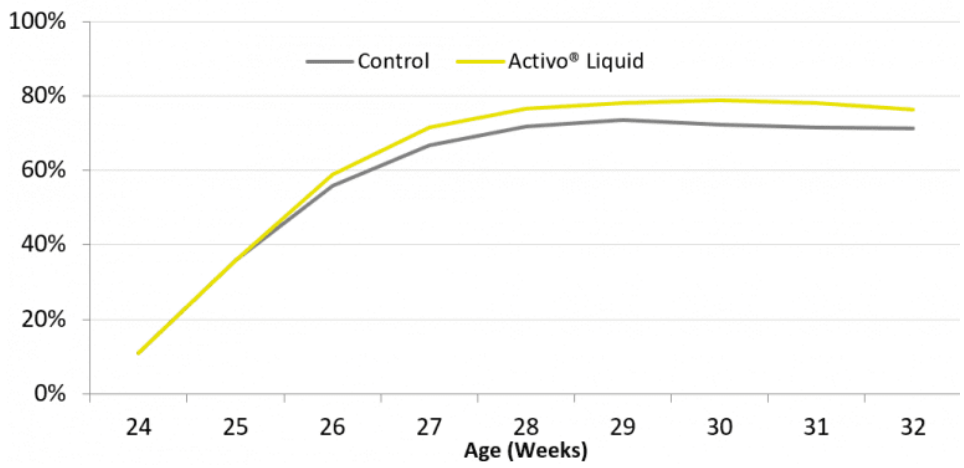

Figure 1: Laying rate (%) of breeder hens during first 9 weeks of production

Study II: Effect of phytomolecules on laying performance after peak production

For a second study, conducted in the Czech Republic, 800 female and 80 male Hubbard breeders (JA57 and M77, respectively) were divided into 2 groups with 5 replicate pens and 80 female and 8 male breeders per pen. The experiment started after the peak-production period, at 34 weeks of age and ended at 62 weeks of age. All animals received a standard mash diet. For one group a phytogenic premix (Activo, EW Nutrition GmbH) was added to the diet at a rate of 100g/MT.

The results indicate that Activo helped maintain the breeder hens' egg laying performance close to the breed's genetic potential (Figure 2). In the course of the experiment, Activo supplemented birds produced 3.6 more eggs than control birds, while consuming a similar amount of feed. As a result, feed consumption per egg produced was lower for birds receiving phytomolecules than for the control birds (169.9 versus 173.6g/d, respectively).

As hatchability was not influenced by the dietary treatment in this study ($P>0.5$), the 3.6 extra eggs resulted in 2.9 extra day old chicks per hen produced, during the post-peak period alone.

The microencapsulated, selected phytomolecules contained in Activo are likely to have improved gut health and feed digestibility, and thereby enhanced the animals' feed efficiency.

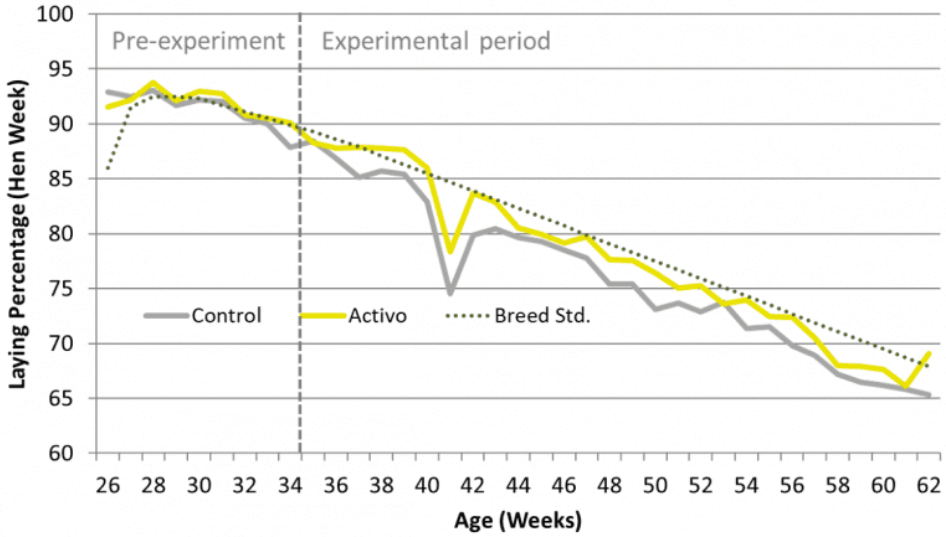

Figure 2: Laying rate (%) of breeder hens week 35 till 62

Chicken or egg? Antibiotic-free poultry production looks at the bigger picture

To successfully produce antibiotic-free poultry meat requires a systematic re-think of each component of the production process. Broiler breeders' lay the foundation for their progeny's health and performance via the egg. Breeder hens need to be in optimal health to consistently deliver optimal eggs. Without recourse to antibiotics for maintaining or recovering intestinal functionality, an effective ABF production needs to make gut health central to its feeding approach.

The trials reviewed demonstrate that selected phytomolecules quantifiably boost breeders' laying performance, increasing the number of hatching eggs and DOCs, while reducing mortality and feed consumption per egg produced. As part of an intelligent antibiotic reduction strategy, the right phytogenic products can be potent tools to help poultry producers achieve their NAE objectives.

by T. van Gerwe, Global Technical Director, and M. Caballero, Global Technical Manager Poultry, EW Nutrition

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Phytomolecules: A tool against antibiotic-resistant E. coli

Diseases caused by E. coli entail use of antibiotics in animal production

E. coli infections are a major problem in pig production. Especially young animals with an incompletely developed immune system are often unable to cope with the cavalcade of pathogens. In poultry, E. coli are responsible for oedema, but also for respiratory diseases. In young piglets, E. coli cause diarrhoea , oedema, endotoxic shock and death. In order to cure the animals, antibiotics often must be applied. Besides this curative application, antibiotics were and in many countries still are used prophylactically and as growth promoters.

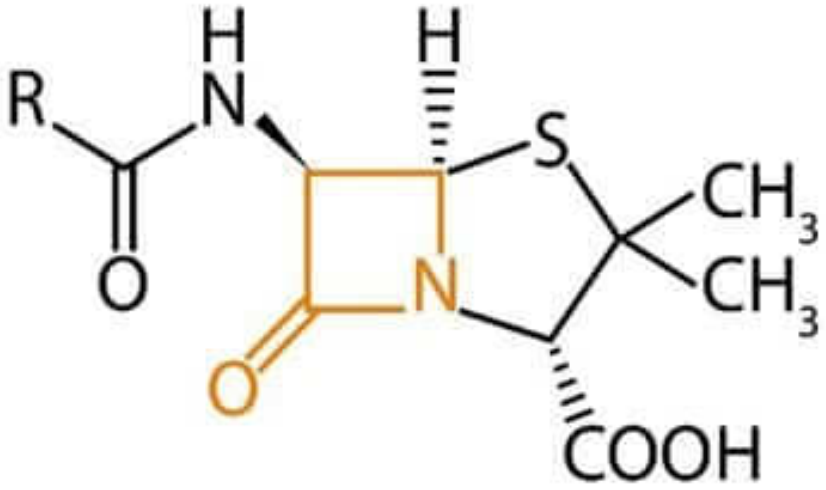
The excessive use of antibiotics, however, leads to the occurrence of antimicrobial resistance (AMR): due to mutations, resistance genes are created which enable enterobacteria such as Salmonella, Klebsiella and E. coli to produce enzymes (ß-lactamases) in order to withstand ß-lactam antibiotics. In case of an antibiotic treatment, the resistant bacteria survive whereas the other bacteria die.
The major problem here is that these resistance genes can be transferred to other bacteria. Harmless

bacteria can thus transfer resistance genes to dangerous pathogens, which then cannot be combatted with antibiotics anymore. In this article we explore in detail how AMR happens and how phytomolecules, which have antimicrobial properties, could be a key tool to reduce the need for antibiotics in animal production.

How ß-lactam antibiotics work

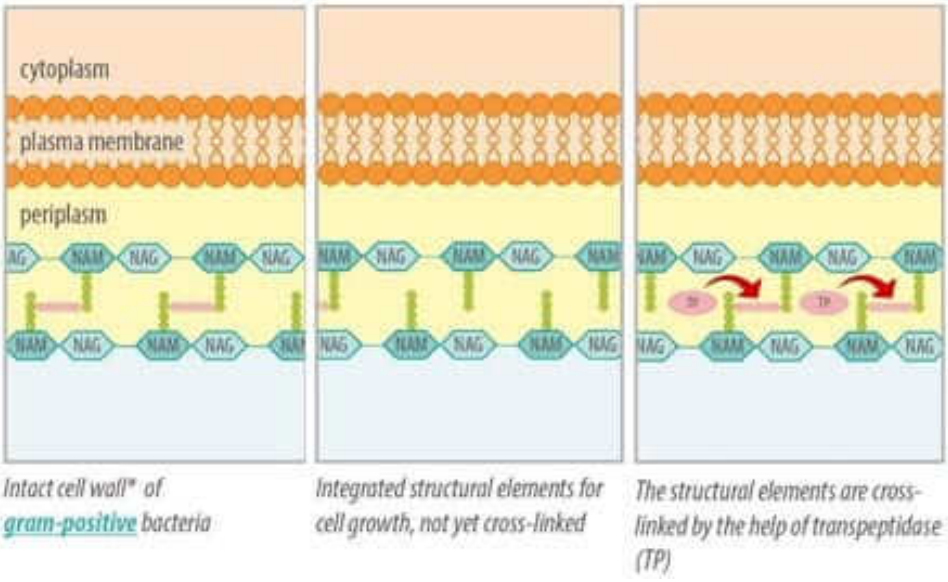
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics are characterised by their lactam ring (Figure 1).

Figure 1: An antibiotic with a ß-lactam ring (in orange)



If bacteria are growing, the cell wall also has to grow. For this purpose existing conjunctions are cracked and new components are inserted. In order for the cell wall to remain a solid barrier, the new components must be interconnected by crosslinks. For the creation of these crosslinks an enzyme is essential, the transpeptidase (figure 2).

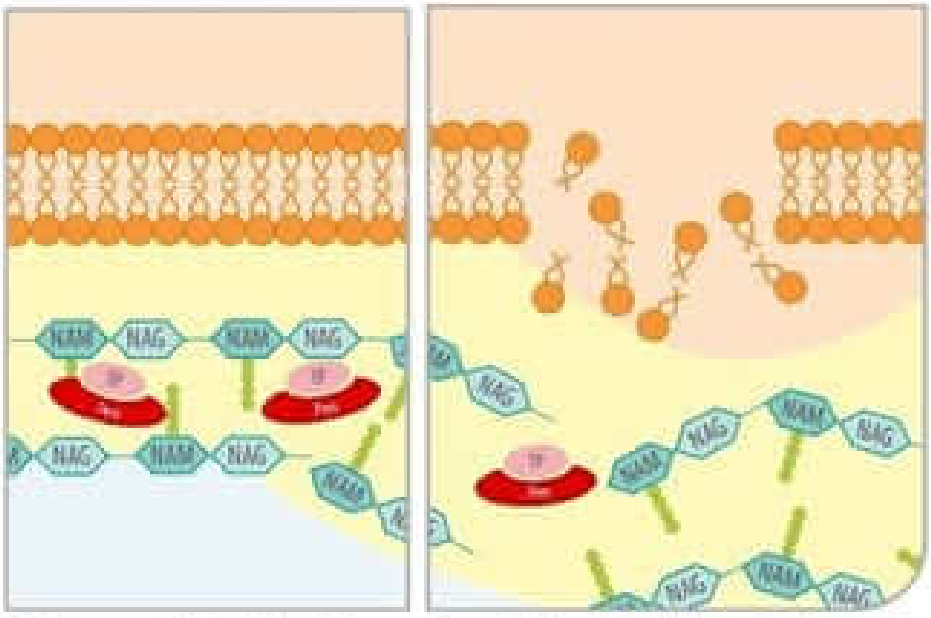
Figure 2: building up a stable cell wall with the help of transpeptidase



Due to their structure, ß-lactam-antibiotics also fit as binding partner for transpeptidase. They bind to the enzyme and block it (Kohanski et al., 2010). The crosslinks cannot be created and the stabilization of the cell wall is prevented. Disturbance of cell wall stability leads to the death of the bacterial cell, hence ß-

lactam antibiotics act bactericidal.

Figure 3: blocked by ß-lactam antibiotics, transpeptidase cannot serve as enzyme for building the cell wall



The challenge: E. coli producing ß-lactamases

Resistant bacteria, which are able to produce ß-lactamases – enzymes that destroy the ß-lactam ring – prevent their own destruction. Divers point mutations within the ß-lactamase genes lead to the occurrence of “extended-spectrum-beta-lactamases“ (ESBL). ESBL are able to inactivate most of the ß-Lactam-antibiotics.

Another mutation leads to so-called AmpC (aminopenicillin and cephalosporin) ß-lactamases. They enable the *E. coli* to express a resistance against penicillins, cephalosporins of the second and third generation as well as against cephamycins.

Phytomolecules – an alternative?

One approach to reduce the use of antibiotics is the utilization of phytomolecules. These secondary metabolites are produced by plants to protect themselves from moulds, yeasts, bacteria and other harmful organisms.

The use of plants and their extracts in human and veterinary medicine is well-established for centuries. Besides digestive and antioxidant characteristics they are well known for their bacteriostatic and bactericidal effects.

Consisting of a high number of chemical compounds, they attack at diverse points and their antimicrobial effect is not caused by only one single specific mechanism. This is crucial because it is therefore very unlikely that bacteria can develop resistances to phytomolecules like they do to antibiotics.

How phytomolecules work

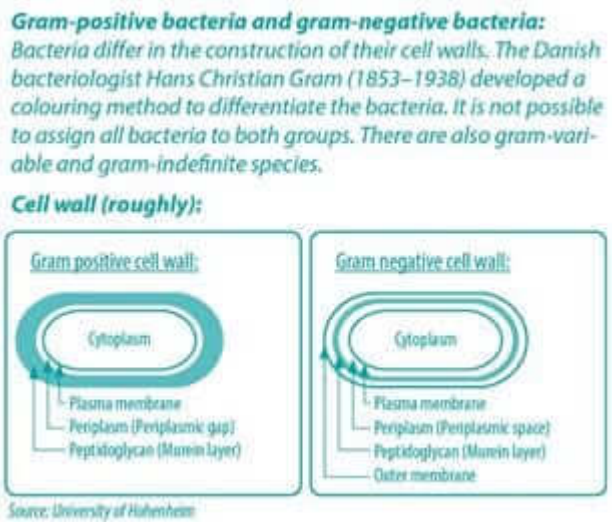
Mostly, phytomolecules act at the cell wall and the cytoplasm membrane level. Sometimes they change the whole morphology of the cell. This mode of action has been studied extensively for thymol and

carvacrol, the major components of the oils of thyme and oregano.

They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as the energy carrier ATP (Adenosin-tri-phosphate). It leads to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

A special challenge: gram-negative bacteria

Gram-negative bacteria such as *E. coli* and *Salmonella* pose a special challenge. The presence of lipopolysaccharides in the outer membrane (OM) provides the gram-negative bacteria with a hydrophilic surface (Nikaido, 2003; Nazarro et al., 2013) (*see also blue infobox*).



The cell wall therefore only allows the passage of small hydrophilic solutes and is a barrier against macromolecules and hydrophobic compounds such as hydrophobic antibiotics and toxic drugs. The bypassing of the OM therefore is a prerequisite for any solute to exert bactericidal activity toward gram-negative bacteria (Helander et al., 1998).

Based on their trial results Helander et al. (1998) (1998) concluded that trans-cinnamaldehyde and partly also thymol and carvacrol gain access to the periplasm and to the deeper parts of the cell. Nikaido (1996) also concluded that OM-traversing porin proteins allow the penetration of lipophilic probes at significant rates.

Evaluating phytomolecules I – in vitro trial, Scotland

A trial conducted in Scotland evaluated the effects of Activo Liquid, a mixture of selected phytomolecules and citric acid, on ESBL-producing *E. coli* as well as on *E. coli* that generate AmpC.

Material and methods

For the trial two strains for each group were isolated from the field, a non-resistant strain of *E. coli* served as control. Suspensions of the strains with 1×10^4 CFU/ml were incubated for 6-7 h at 37°C (98.6°F) together with diverse concentrations of Activo Liquid or with cefotaxime, a cephalosporin. The cefotaxime group saved as a control for differentiating resistant and non-resistant *E. coli*.

The suspensions were put on LB agar plates and bacteria colonies were counted after further 18-22h incubation at 37°C.

Results

The antimicrobial efficacy of the blend of phytomolecules depended on the concentration at which they were used (see table 1). A bacteriostatic effect could be shown at dilutions up to 0.1 %, a bactericidal effect at higher concentrations.

Table 1: Effect of phytomolecules against resistant E. coli producing ESBL and AmpC in poultry

Poultry Microbiology Laboratory, Edinburgh, Scottland	Cefotaxime	Phytomolecules (Activo® Liquid)			
	30 µg / ml	0.1 %	0.2 %	0.4 %	0.5 %
E. coli ESBL 1 (Poultry)	-	+	++	++	++
E. coli ESBL 2 (Poultry)	-	+	++	++	++
E. coli AmpC 1 (Poultry)	-	+	++	++	++
E. coli AmpC 2 (Poultry)	-	++	++	++	++
E. coli non-resistant	+	+	++	++	++

\- no effect
\+ growth inhibiting (bacteriostatic)
++ bactericidal

Evaluating phytomolecules II – in vitro trial, Germany

A further trial was conducted in Germany (Vaxxinova, Münster), confirming the preceding results.

Material and methods

Four ESBL producing *E. coli* all isolated from farms and a non-resistant reference strain as control were tested concerning their sensitivity against Activo Liquid. Every bacteria strain (Conc.:1×10^4 CFU/ml) was subjected to a bacterial inhibition assay in an appropriate medium at 37°C for 6-7 hours.

Results

In this trial Activo Liquid also showed a dose-dependent efficacy, with no or just a bacteriostatic effect up to a concentration of 0.1 %, but bactericidal effects at a concentration of ≥0.2 % (table 2).

Table 2: Effect of phytomolecules against resistant ESBL producing E. coli in pig and in poultry

Vaxxinova GmbH, Münster	Phytomolecules (Activo® Liquid)			
	0.1 %	0.2 %	0.4 %	1 %
E. coli ATCC25922	+	++	++	++
E. coli ESBL 1 (Pig)	-	++	++	++
E. coli ESBL 2 (Pig)	+	++	++	++
E. coli ESBL 3 (Poultry)	+	++	++	++
E. coli ESBL 4 (Poultry)	-	++	++	++

\- no effect
\+ growth inhibiting (bacteriostatic)
++ bactericidal

Phytomolecules: a promising outlook

E. coli infections have devastating effects on animals, from diarrhea to edema, enterotoxic shock and even death. Antibiotic treatments have long been the only practicable answer. However, their excessive use – for instance, the metaphylactic application to thousands of animals in a flock – has led to the development of resistant strains. There is evidence that a reduction of antibiotic use reduces the occurrence of resistances (Dutil et al., 2010).

The results of the two in vitro trials in Scotland and Germany demonstrate the bactericidal effects of phytomolecules on *E. coli* that produce ESBL and AmpC. Using phytomolecules could thus reduce the use of antibiotics and therefore also the occurrence of AMR.

While it is theoretically possible for bacteria to also become resistant against phytomolecules, the probability of this happening is very low: unlike antibiotics, phytomolecules contain hundreds of chemical components with different modes of action. This makes it exceedingly difficult for bacteria to adapt and develop resistance. To tackle the problem of antibiotic-resistant *E. coli*, antimicrobial phytomolecules therefore offer a promising, sustainable and long-term solution.

By Dr. Inge Heinzl, Editor, EW Nutrition

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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

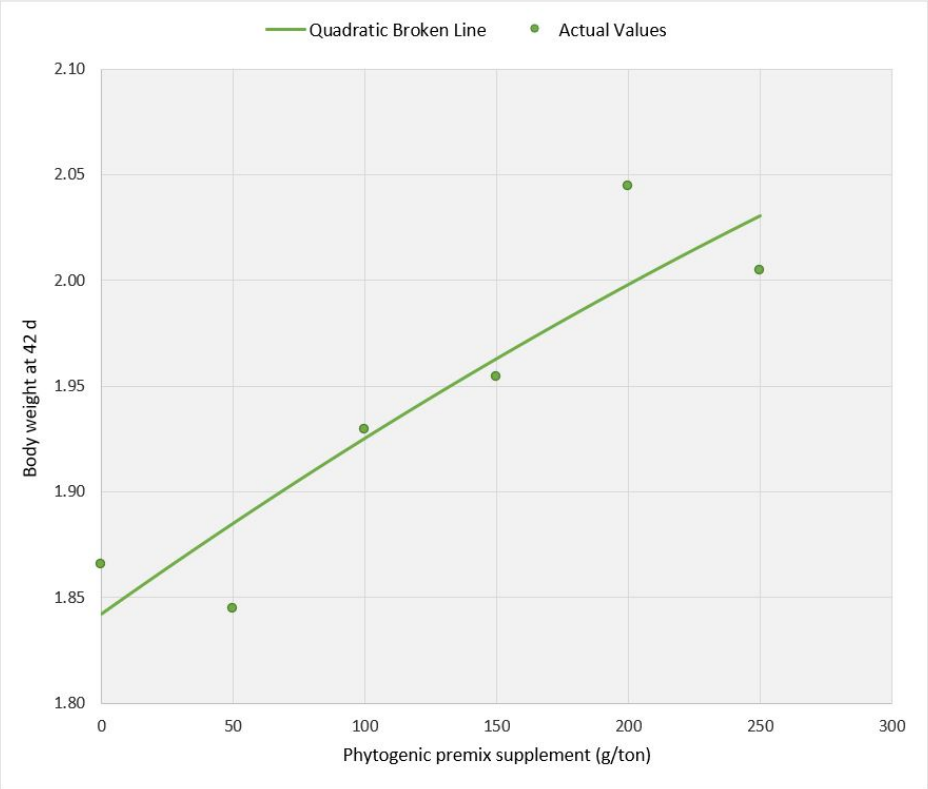
Results: Inclusion levels from 100 mg/kg of Activo onwards improved laying performance, egg mass and egg weight and reduced FCR compared to the control group. Results recorded for hematological parameters were within the reference range of healthy birds when feeding up to 5.000 mg Activo/ kg of feed.

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

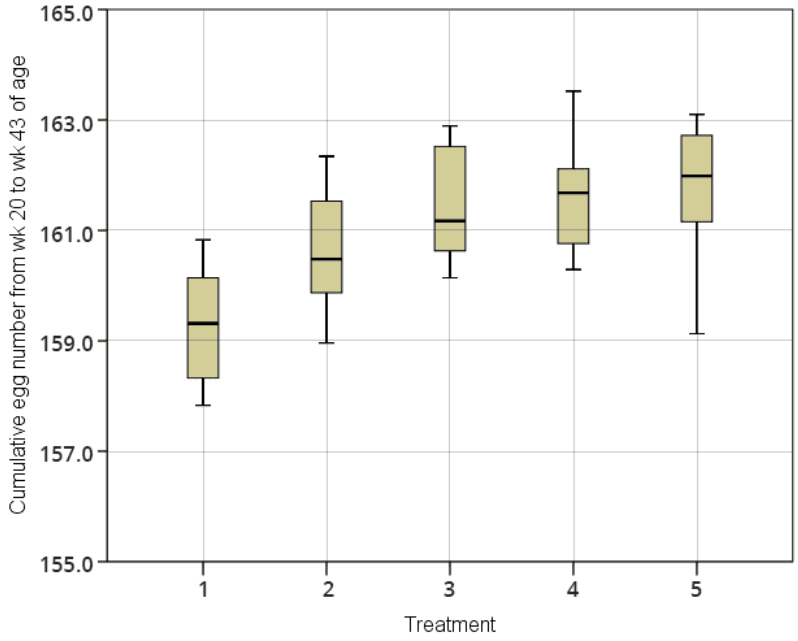
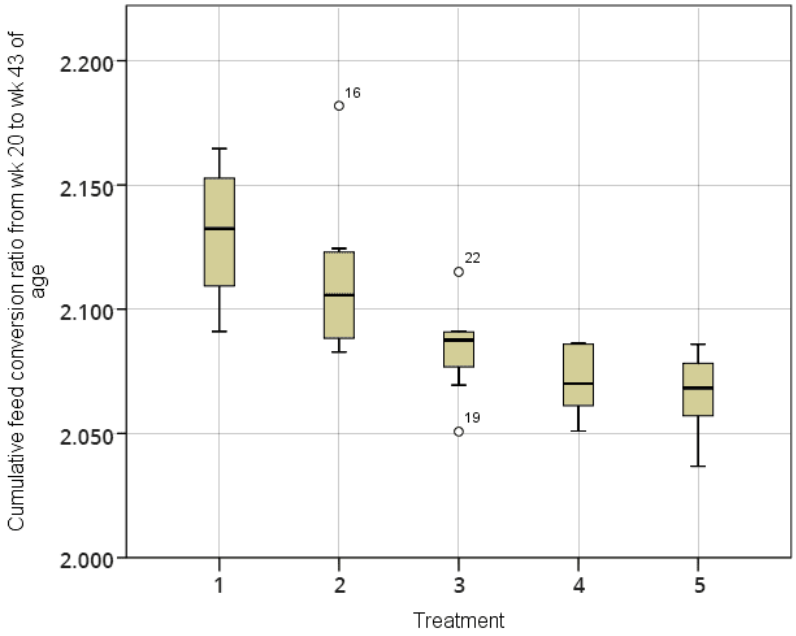


Figure 3: Dose-dependent effects of Activo on feed efficiency in laying hens



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

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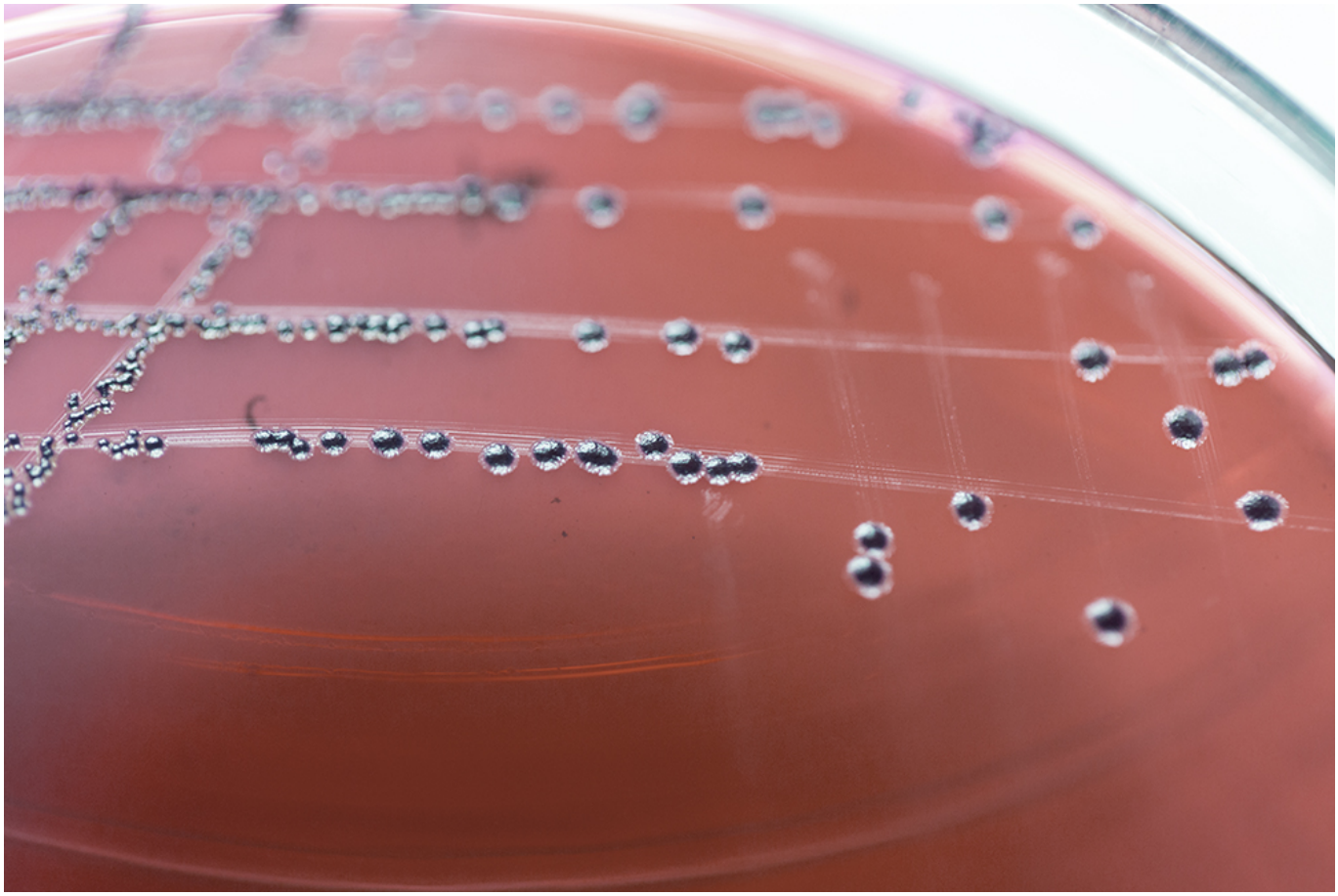
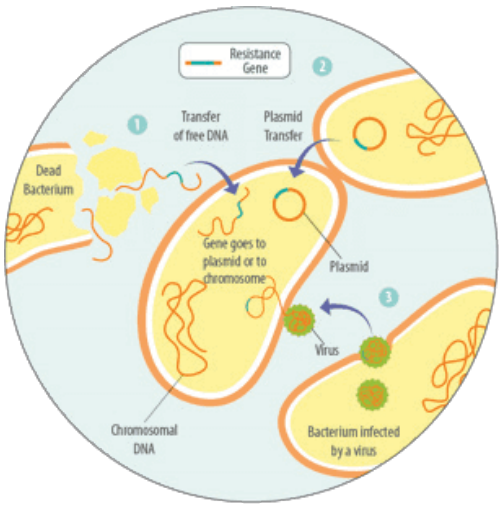
Photo source: Aviagen

Secondary Plant Compounds (SPC's) to reduce the use of antibiotics?



Initial in vitro trials give reason for hope

Antibiotic Resistance



Some bacteria, due to mutations, are less sensitive to certain antibiotics than others. This means that if certain antibiotics are used, the insensitive ones survive. Because their competitors have been eliminated, they are able to reproduce better. This resistance can be transferred to daughter cells by means of „resistance genes“. Other possibilities are the intake of free DNA and therefore these resistance genes from dead bacteria 1, through a transfer of these resistance genes by viruses 2 or from other bacteria by means of horizontal gene transfer 3 (see figure 1). Every application of antibiotics causes a selection of resistant bacteria. A short-term use or an application at a low dosage will give the bacteria a better chance to adapt, promoting the generation of resistance (Levy, 1998).

Antibiotics are promoting the development of resistance:

- Pathogenic bacteria possessing resistance genes are conserved and competitors that do not

possess these genes are killed
- Useful bacteria possessing the resistance genes are conserved and serve as a gene pool of antibiotic resistance for others
- Useful bacteria without resistance, which probably could keep the pathogens under control, are killed

Reducing the use of antibiotics
Ingredients from herbs and spices have been used for centuries in human medicine and are now also used in modern animal husbandry. Many SPC's have antimicrobial characteristics, e.g. Carvacrol and Cinnamon aldehyde. They effectively act against Salmonella, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Entero– and *Staphylococcus,* and *Candida albicans*. Some compounds influence digestion, others act as antioxidants. Comprehensive knowledge about the single ingredients, their possible negative but also positive interaction (synergies) is essential for developing solutions. Granulated or microencapsulated products are suitable for addition to feed, liquid products would be more appropriate for an immediate application in the waterline in acute situations.

	(Activo® Liquid)		
Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)	10%	2%	1%
E. coli (reference strains)	++	+	+
Proteus vulgaris (reference strains)	+	+	+
Pseudomonas fluorescens	++	+	-
Salmonella pulmorum	++	++	+
Salmonella gallinarum	++	++	+
Staphylococcus aureus (reference strains)	+++	++	++

+++	22 – 29 mm ZOI (zone of inhibition)
++	15 – 21 mm ZOI
+	10 – 14 mm ZOI
-	< 10 mm ZOI

SPC's (Activo Liquid) against livestock pathogens in vitro
In "agar diffusion tests", the sensitivity of different strains of farm-specific pathogens was evaluated with different concentrations of Activo Liquid. The effectiveness was determined by the extent to which they prevented the development of bacterial overgrowth. The larger the bacteria-free zone, the higher the antimicrobial effect.

In this trial, Activo Liquid showed an antimicrobial effect on all bacteria tested. The degree of growth inhibition positively correlated with its concentration.

Table 1: Inhibition of field isolated standard pathogens by different concentrations of Activo Liquid

Activo Liquid against antibiotic resistant field pathogens in vitro

It cannot be excluded that resistant pathogens not only acquired effective weapons to render antibiotics harmless to them but also developed general mechanisms to rid themselves of otherwise harmful substances. In a follow-up laboratory trial, we evaluated whether the Activo Liquid composition is as effective against ESBL producing E. coli and Methicillin resistant S. aureus (MRSA) as to non-resistant members of the same species.

	SPC's (Activo® Liquid)			
Laboratory: Vaxxinova, Muenster, Germany	0.1%	0.2%	0.4%	1%
E.coli reference ATCC25922	+	++	++	++
ESBL 1 (Pig)	-	++	++	++
ESBL 2 (Pig)	+	++	++	++
ESBL 3 (Poultry)	+	++	++	++
ESBL 4 (Poultry)	-	++	++	++
S. aureus reference ATCC29213	-	+	+	++
MRSA 1 (Pig)	-	+	+	++
MRSA 2 (Pig)	-	+	+	++

-	no effect
+	growth inhibiting
++	bactericide

Trial Design: Farm isolates of four ESBL producing E. coli and two MRSA strains were compared to nonresistant reference strains of the same species with respect to their sensitivity against Activo Liquid. In a Minimal Inhibitory Concentration Assay (MIC) under approved experimental conditions (Vaxxinova Diagnostic, Muenster, Germany) the antimicrobial efficacy of Activo Liquid in different concentrations was evaluated.

The efficacy of SPC's (Activo Liquid) against the tested strains could be demonstrated in a concentration-dependent manner with antimicrobial impact at higher concentrations and bacteriostatic efficacy in dilutions up to 0,1% (ESBL) and 0,2% (MRSA)(table 2).

Conclusion:

To contain the emergence and spread of newly formed resistance mechanisms it is of vital importance to reduce the use of antibiotics. SPC's are a possibility to decrease antibiotic use especially in pro- and metaphylaxis, as they show good efficacy against the common pathogens found in poultry, even against resistant ones.

I. Heinzl

Necrotic enteritis in poultry

Enteric diseases cause significant economic losses due to decreased weight gain, higher mortality, higher feed conversion, higher veterinary costs and medicine and a higher risk of contamination by poultry products in food production. The losses due to necrotic enteritis mainly occurring in broilers and fattening turkeys in intensive floor or free-range management are put at 2 billion US$ per year.

After the ban of antibiotic growth promoters, the relevance of this formerly well controllable disease reappeared and increased.

Necrotic enteritis is a disease of the gut
It is caused by specific gram-positive, anaerobic bacteria – *Clostridium perfringens,* mostly Type A. Clostridia are found in litter, faeces, soil, dust and in healthy animals' guts. These spore forming bacteria are extremely resistant against environmental influences and can survive in soil, feed, and litter for several years and even reproduce.
Clostridium perfringens is a component of the normal gut flora. It occurs in a mixture of diverse strains in a concentration of up to 10^5 CFU / g intestinal content. In animals suffering from necrotic enteritis particularly one strain of *Clostridium perfringens* is found in a much more higher concentration of 10^6-10^8 CFU / g.
Necrotic enteritis affects chickens and turkeys at the age of 2-16 weeks, proliferating at the age of 3-6 weeks. There is an acute clinical, and a subclinical form.
Birds suffering from the clinical form clearly show symptoms like a poor general state of health and diarrhoea. Mortality rates up to 50 % can occur. Subclinical necrotic enteritis cannot be diagnosed easily, as there are no clear symptoms. This form, however, stays within the flock and causes losses due to decreased growth.

Factors promoting an infection with necrotic enteritis should be avoided!
In general, factors have to be cited that create an intestinal environment favourable for the facultative anaerobic *Clostridium perfringens* or weaken the immune status of the host:

1. **Feed:**
   Here **NSP's** have to be mentioned. Undigested NSP's serve as substrate and some of them cause higher production of mucus also serving as substrate and providing ideal anaerobic conditions. Undigested **proteins** due to high contents in the diet also serve as substrates. **Animal protein** and **fat** are worse than vegetable variants and a homogeneous size of particles in the diet is better than an **inhomogeneous mixture**.
2. **Stress**
   Stresses such as feed change or high stocking density favour NE
3. **Diseases**
   Immunosuppressive diseases such as infective chicken anaemia, Gumboro or Marek's decrease resistance against intestinal infections and facilitate their colonisation. Some pathogens exert pressure on the gut and prepare the way for clostridia. Here Cryptosporidia and salmonella have to be mentioned.

New approaches
Secondary plant compounds show good results against the two microorganisms just mentioned. In a trial conducted with free range broilers in France, a combination of a vaccination against coccidia and a mixture of secondary plant compounds (Activo liquid) resulted in a reduced occurrence of necrotic enteritis in the trial group compared to the control. Additionally due to an improved feed conversion, the margin per animal in the trial group was 5 Cent higher than in the control (1,44 € vs 1,39 €).
In an *in vitro* test, Activo liquid also showed bactericidal efficacy against field isolated *Salmonella pulmorum* and *Salmonella gallinarum* at a 2 % concentration.
The trials show that combined with a good feeding and stress management, secondary plant compounds, could be a good tool to eliminate predisposing factors for necrotic enteritis and could therefore help control this economically important disease.

Secondary plant compounds are the new frontier in poultry nutrition

Why should you read another story about phytogenics? Or, is it botanicals, spices, herbs, and extracts? No matter what we call them, scientists have named them "secondary plant compounds", and if we are to follow the American tradition we can call them SPC. Then, here is the first interesting thing we can discuss about this plant-derived class of active compounds. They are "secondary" in nature, but not insignificant. They play no role in normal metabolism, but they help plants (and now animals) survive under adverse conditions. Perhaps, this is why some experts consider them as the next frontier in poultry nutrition. With poultry that are raised in less than ideal conditions, especially when we consider the movement towards antibiotic reduction (for growth promoting reasons, not complete removal of all medicines), we understand that such natural compounds can be of significant help.

As it happens, the majority of poultry specialists in Europe and increasingly in the Americas consider SPC as an almost-essential element in diets for broilers and layers (and turkeys, ducks, and all poultry for that matter) when birds are raised without antibiotics. Some go even further and use them along with antibiotics because, as we all know, antibiotics are never 100% efficient as bacteria sooner or later develop some form of resistance. Such resistance has not yet been observed with SPC. So if one is to use SPC in poultry feds, which ones to buy? A quick glance at the market will reveal more commercial products than can possibly be imagined. Some must be better than the rest, but how can we separate the wheat from the chaff? Price alone is not always a good indicator. A high quality product must be expensive – for there is no such thing as a free lunch – but all expensive products are not always of the highest possible quality!

There are three basic criteria, which we can mention briefly here:

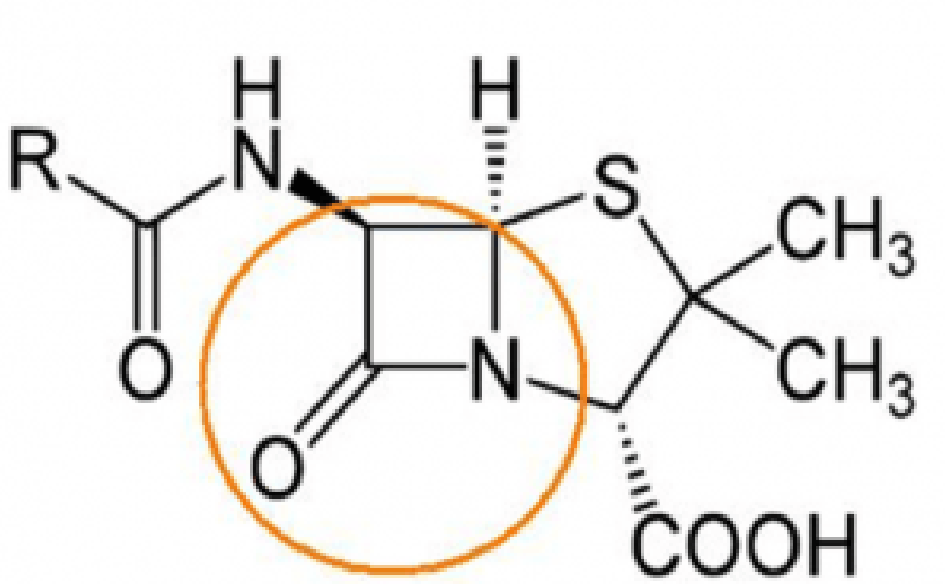
1. **SPC are volatile** – at least most of them. As such, unprotected products will soon evaporate if left in the open air as it happens with feed prepared in commercial farms. So, some form of protecting SPC is essential.
2. **SPC are innumerable** – so finding the right mix for the job required is important. You cannot get the same results with any kind of mix. So, in designing an SPC mix, the manufacturer must declare and have knowledge of the target to be accomplished.
3. **SPC are powerful** – meaning you cannot just keep adding as much as possible. Here finding the exact dosage for the right purpose is a difficult balancing exercise. So, the right mix and the right dosage must be combined, otherwise animals will refuse the feed (worst case scenario) or just fail to benefit from SPC inclusion.

There is so much more to learn about this exciting class of compounds that can replace the growth promoting action of antibiotics that it is worth spending time learning more about them.

Secondary plant compounds against antibiotic-resistant E. coli

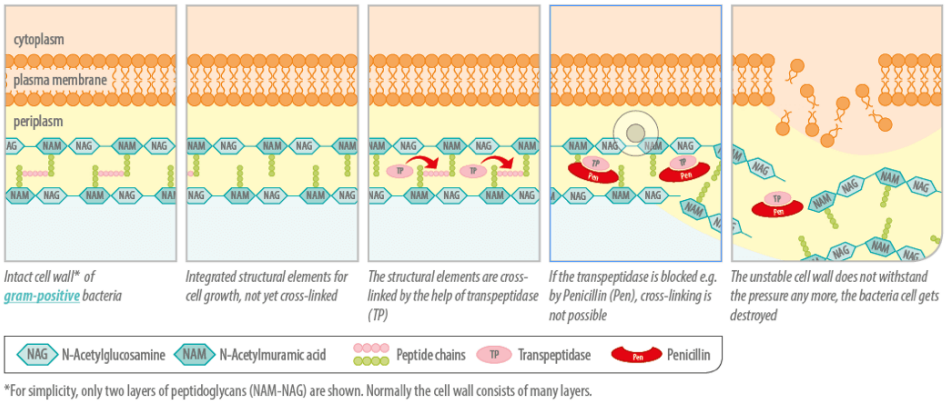
Due to incorrect therapeutic or preventive use of antibiotics in animal production as well as in human medicine, occurrence of antibiotic resistant pathogens has become a widespread problem. Enterobacteria in particular (e.g. Salmonella, Klebsiella, E. coli) possess a special mechanism of resistance. By producing special enzymes (ß-lactamases), they are able to withstand the attack of so-called ß-lactam antibiotics. The genes for this ability (resistance genes) can also be transferred to other bacteria resulting in a continuously increasing problem. Divers point mutations within the ß-lactamase genes lead to the occurrence of „Extended-Spectrum-Beta-Lactamases“ (ESBL), which are able to hydrolyse most of the ß-Lactam-antibiotics. AmpC Beta-Lactamases (AmpC) are enzymes, which express a resistance against penicillins, cephalosporins of the second and third generation as well as cephamycins.

What are ß-lactam antibiotics?
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams and carbapenems. A characteristic of these antibiotics is the lactam ring (marked in orange):



Mode of action of ß-lactam antibiotic
If a bacterial cell is growing, the cell wall also has to grow. For this purpose, existing conjunctions are cracked and new components are inserted. ß-lactam-antibiotics disturb the process of cell wall construction by blocking an enzyme needed, the transpeptidase. If crosslinks necessary for the stability of the cell wall cannot be created, the bacteria cannot survive. Resistant bacteria, which are able to produce ß-lactamases, destroy the ß-lactam antibiotics and prevent their own destruction.

Secondary plant compounds
Secondary plant compounds and their components are able to prevent or slow down the growth of moulds, yeasts, viruses and bacteria. They attack at various sites, particularly the membrane and the cytoplasm. Sometimes they change the whole morphology of the cell. In the case of gram-negative bacteria, secondary plant compounds (hydrophobic) have to be mixed with an emulsifier so that they can pass the cell wall which is open only for small hydrophilic solutes. The modes of action of secondary plant compounds depend on their chemical composition. It also depends on whether single substances or blends (with possible positive or negative synergies) are used. It has been observed that extracts of spices have a lower antimicrobial efficacy than the entire spice.



The best explained mode of action is the one of thymol and carvacrol, the major components of the oils of thyme and oregano. They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as ATP leading to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

Trial (Scotland)

Design

Two strains of ESBL-producing and AmpC respectively, isolated from the field, a non-resistant strain of E. coli as control. Suspensions of the strains with 1×10^4 KBE/ml were incubated for 6-7 h at 37°C together with different concentrations of Activo Liquid or with cefotaxime, a cephalosporin. The suspensions were then put on LB-Agar plates and bacteria colonies were counted after a further 18-22h incubation at 37°C. Evaluation of the effects of Activo Liquid on ESBL-producing as well as on E. coli resistant for aminopenicillin and cephalosporin (AmpC)

Results

The antimicrobial efficacy of the blend of secondary plant compounds depended on concentration with bactericidal effect at higher concentrations and bacteriostatic at dilutions up to 0,1%. It is also possible that bacteria could develop a resistance to secondary plant compounds; the probability is however relatively low, due to the fact that essential oils contain hundreds of chemical components (more than antibiotics) making it difficult for bacteria to adapt.