

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.

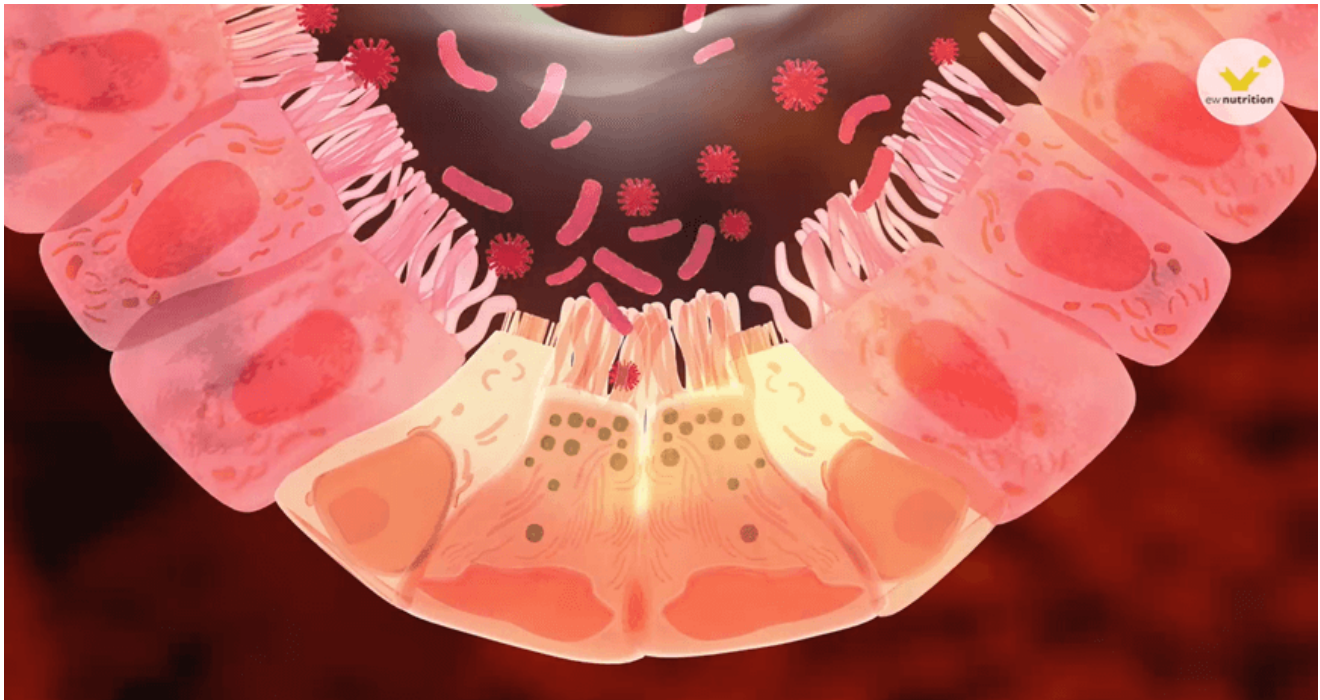
In fact, the right protection, the right mix and the right inclusion rates must be combined to ensure that the animals do not refuse the feed (worst case scenario) or just fail to benefit from the inclusion of phytomolecules.

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

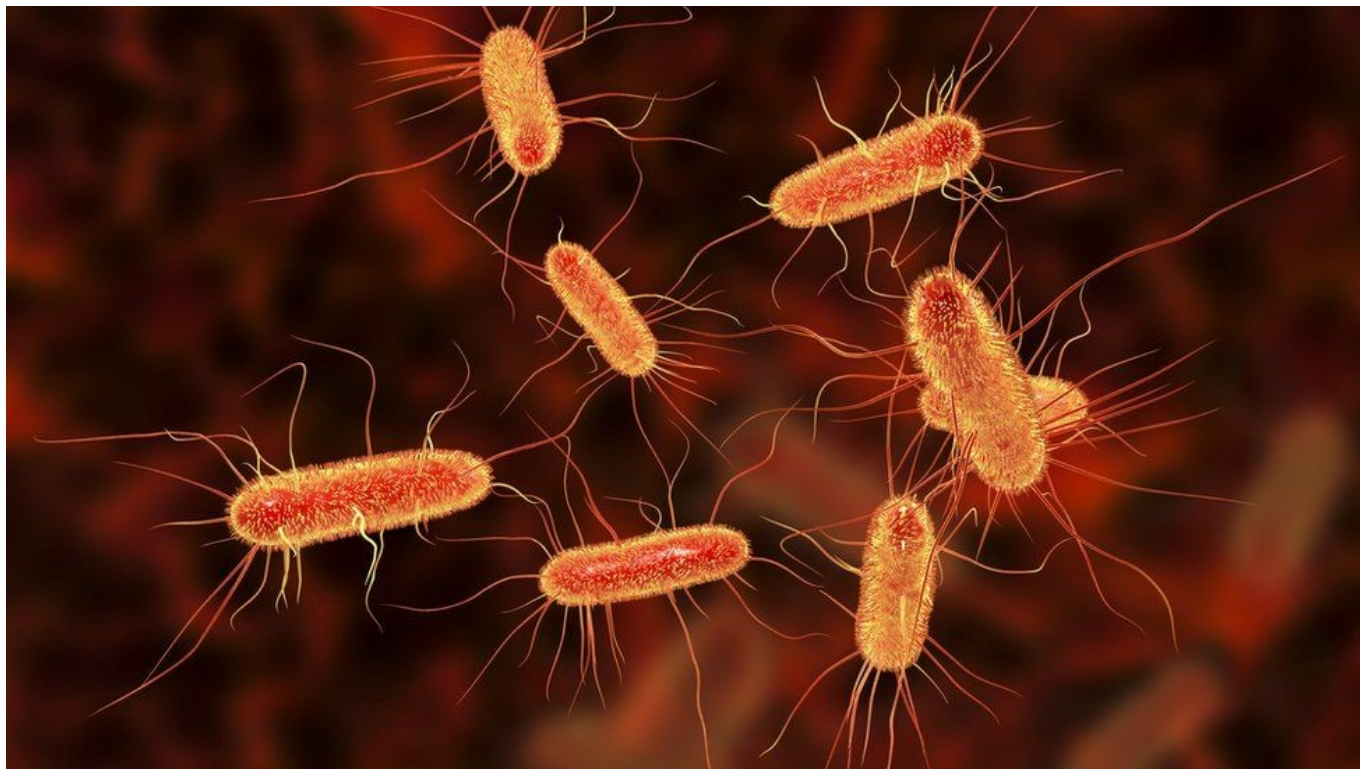
plants as a defense mechanism against pathogens, phytomolecules promote the digestion of feed ingredients (Zhai et al. 2018), prevent loss of gut integrity during enteric challenges (Liu et al. 2018), and have antimicrobial properties that hinder the growth of potential pathogens (Chowdhury, 2018). Phytomolecules 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.*

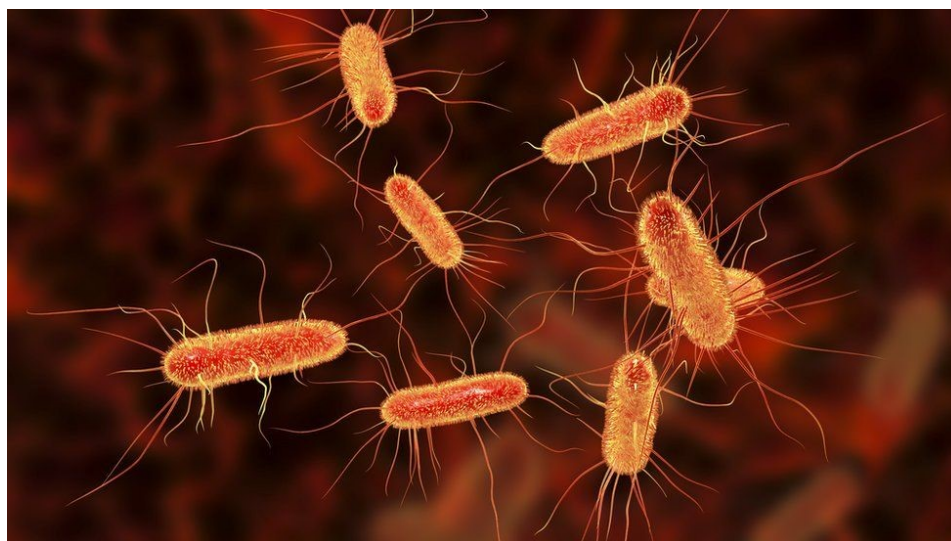


The hidden danger of endotoxins in animal production



Find out more about endotoxins [here](#)

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).

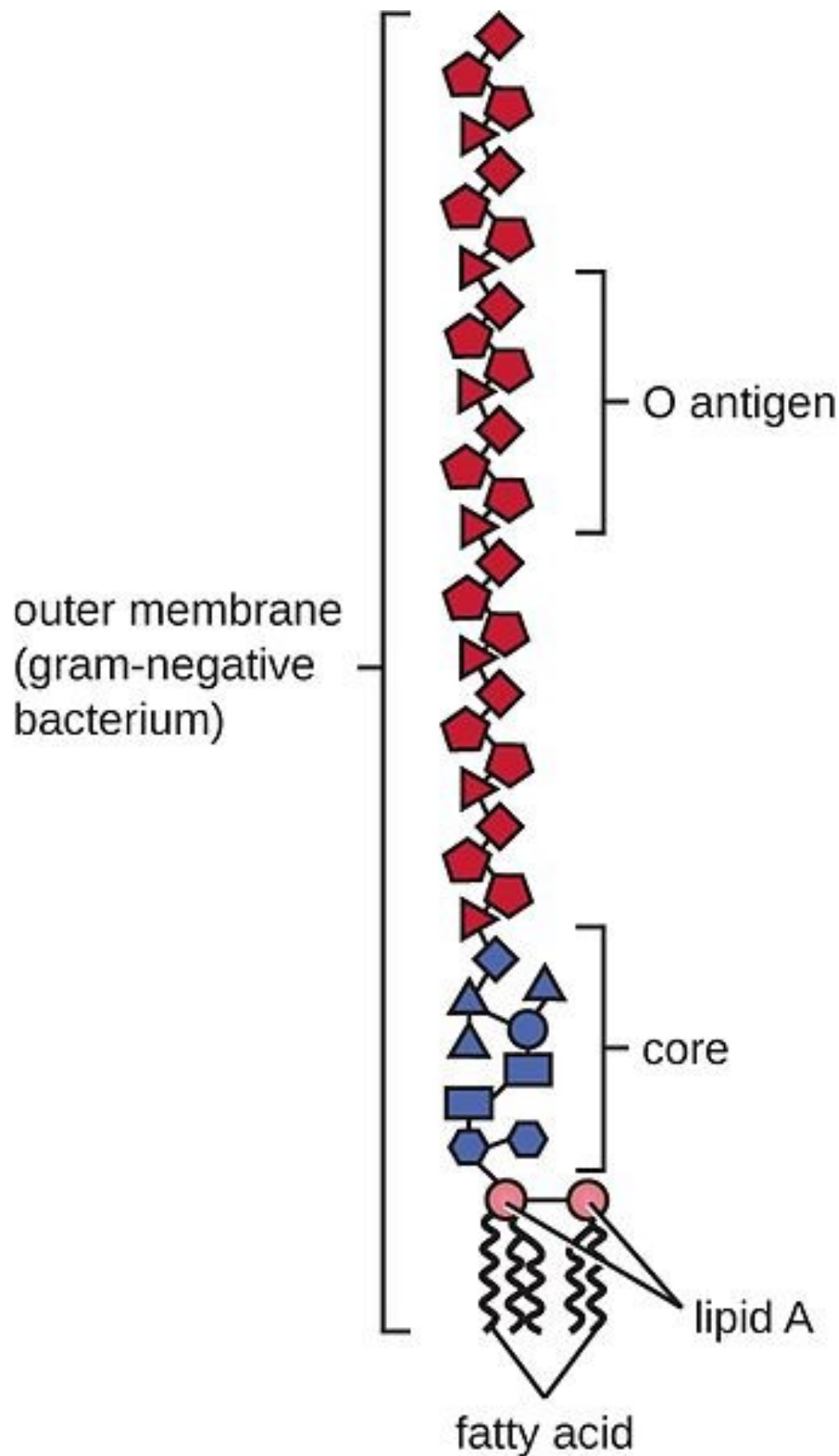


Figure 1: Structure of an LPS

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 $\approx 830 \mu\text{m}$ in

the colon and $\approx 123 \mu\text{m}$ 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-1 β , 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).

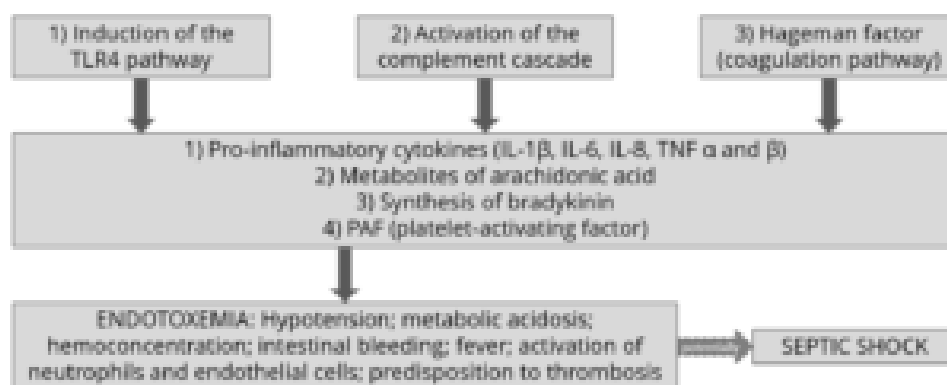


Figure 2: How LPS leads to endotoxemia – 3 modes of action

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 continuously 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.

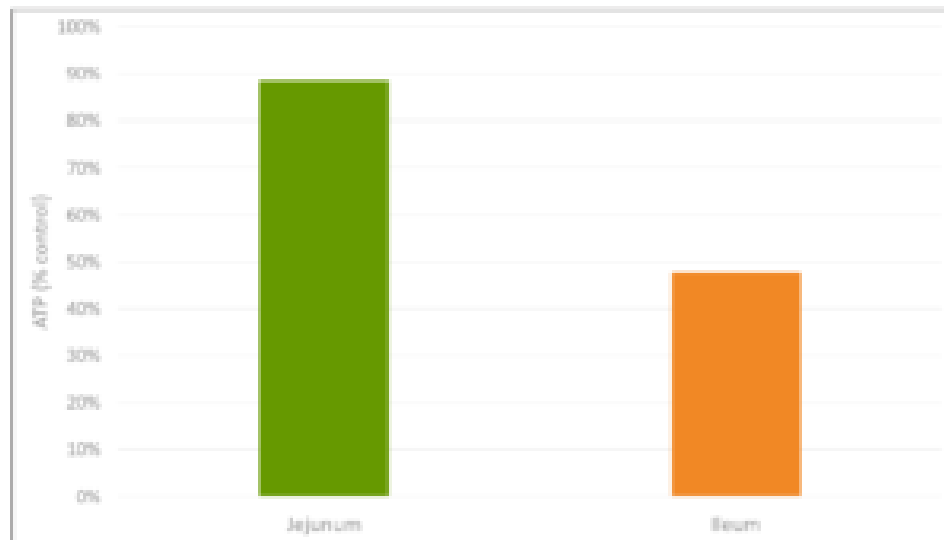


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from [Li et al., 2015](#))

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, CO₂ 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.

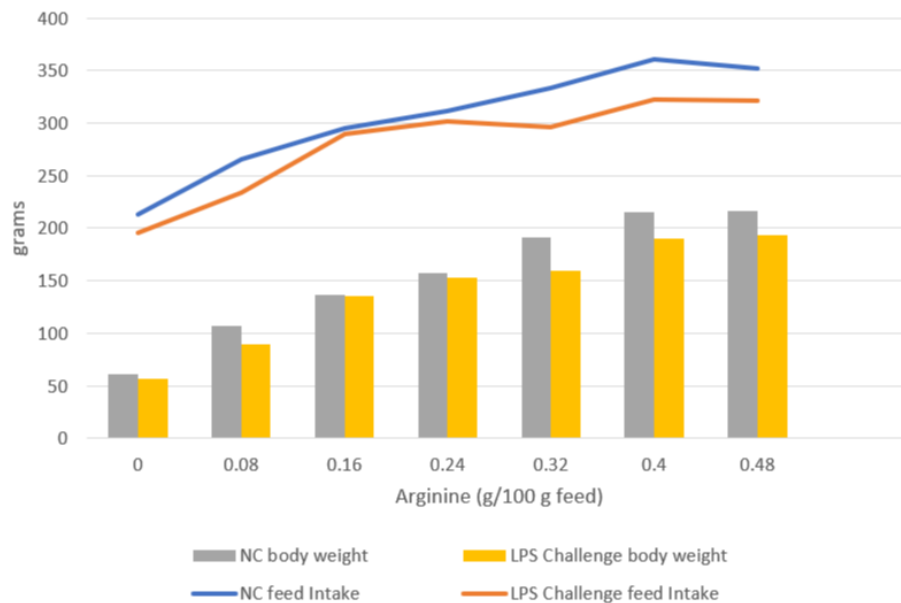


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on [Webel, Johnson, and Baker, 1998](#))

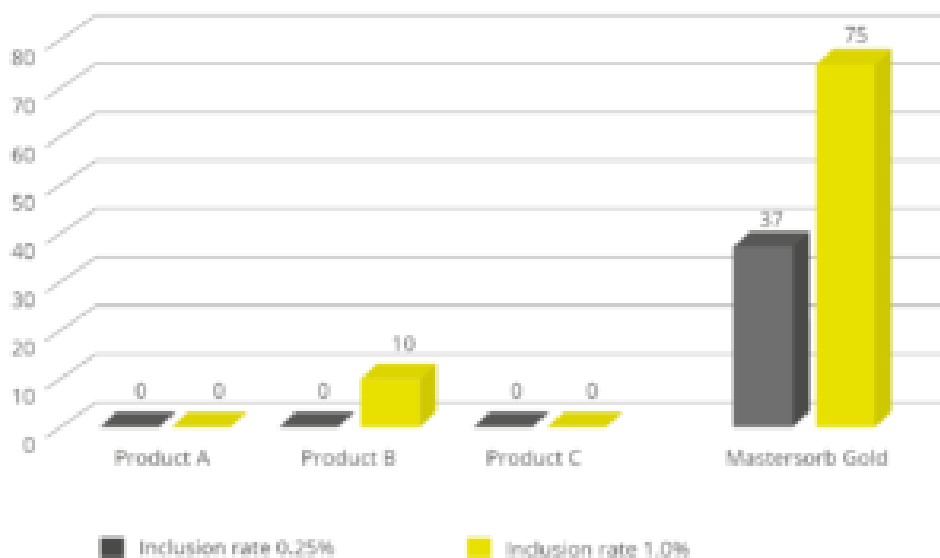
NC = negative control

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 are at great risk of immunosuppression caused by the immune system “overdrive.” Effective endotoxin mitigating agents can help to prevent these scenarios.

EW Nutrition’s Mastersecure 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 Mastersecure 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 Mastersecure Gold reduced the toxin load on the solution by 37%. At 1% inclusion, Mastersecure Gold (noted as Mastersorb below) bound 75% of the toxin, while only one competitor product demonstrated any binding (10%).



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 [MasterSecure 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.

By Claudio Campanelli, EW Nutrition

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How producers keep the egg supply chain going amid COVID-19



The Covid-19 pandemic has increased consumer demand for eggs. This article discusses how the egg supply chain, from layer farms to supermarkets, works amid disruptions caused by Covid-19.

Respiratory Challenges: Breathing Space for Antibiotic Reduction?



Sub-therapeutic doses of antibiotic growth promoters (AGPs) were used for more than 50 years in poultry production to achieve performance targets – until growing concerns arose regarding antibiotic resistance (Kabir, 2009) and decreasing efficacy of antibiotics for medical purposes (Dibner & Richards, 2005).

Isolates of ESBL-producing *E.coli* from animals, farmworkers, and the environment were found to have identical multidrug resistance patterns (A. Nuangmek et al., 2018). There is also evidence that AMR strains of microorganisms spread from farm animal to animal workers and beyond. Global AMR fatalities are increasing and might reach 10 million by 2050 (Mulders et al., 2010, Trung et al., 2017, Huijbers et al., 2014).

In light of this, certain AGPs have already been banned, and there is a strong possibility of future restrictions on their use worldwide. Bans are effective: the MARAN report 2018 shows that lower antibiotics usage following the EU ban on AGPs has reduced resistant *E.coli* in broilers. Another positive consideration is the market opportunities that exist for antibiotic residue-free food.

However, the key element that poultry producers need to get right for antibiotic reduction to be successful is [respiratory health management](#). This article looks at why respiratory health is a particular challenge – and how phytogenic solutions can help.

A closer look at the chickens' respiratory system

The respiratory tract is equipped with a functional mucociliary apparatus consisting of a protective mucous layer, airway surface liquid layer, and cilia on the surface of the ciliated cells. This apparatus produces mucus, which traps the inhaled particles and pathogens and propels them out of the airways. This mechanism, called the mucociliary clearance, is the primary innate defense mechanism of the respiratory

system.

High stocking density combined with stressful environmental factors can negatively influence birds' immune systems (Heckert et al., 2002; Muniz et al., 2006), making them more susceptible to respiratory disease. When a bird suffers from respiratory disease, which is nowadays usually complicated by a co-infection or secondary bacterial infection, there is an excess production of mucus that results in ciliostasis and, therefore, in an impaired mucociliary clearance. The excess mucus in the tract obstructs the airways by forming plaques and plugs, resulting in dyspnea (hypoxia) and allowing the invasive bacteria to adhere and colonize the respiratory system.

The build-up of mucus in the respiratory tract severely reduces oxygen intake, causing breathlessness, reduced feed intake, and a drop in the birds' energy levels, which negatively impacts weight gain and egg production. Respiratory problems can result from infection with bacteria, viruses, and fungi, or exposure to allergens. The resultant irritation and inflammation of the respiratory tract leads to sneezing, wheezing, and coughing – and, therefore, the infection rapidly spreads within the flock.



Relatively high stocking density is the norm in poultry production

Low or no antibiotics: how to manage respiratory disease?

Unsurprisingly, respiratory diseases in poultry are a major cause of mortality and economic loss in the poultry industry. For Complicated Chronic Respiratory Disease (CCRD), for instance, although the clinical manifestations are usually slow to develop, *Mycoplasma gallisepticum* (MG), in combination with *E. coli*, can cause severe airsacculitis. Beside feed and egg production reduction, these problems are of high economic significance since respiratory tract lesions can cause high morbidity, high mortality, and significant carcass condemnation and downgrading.

Producers need to pre-empt the spread of respiratory pathogens, react quickly to alleviate respiratory distress and maintain the mucociliary apparatus' functionality. Traditionally, treatment options are based on antiviral, anti-inflammatory, and antibiotic drugs. Can the poultry industry limit losses from respiratory infections without excessive recourse to antibiotics?

Indeed, a sudden reduction in antibiotic usage comes with a risk of impaired performance, increased mortality, and impaired animal health and welfare. The impact has been quantified as a 5% loss in broiler

meat production per sq. meter (Gaucher et al., 2015). Effective antibiotics reduction requires a combination of innovative products and suitable consultancy services to manage poultry gut health, nutrition, flock management, biosecurity, and, particularly, respiratory health.

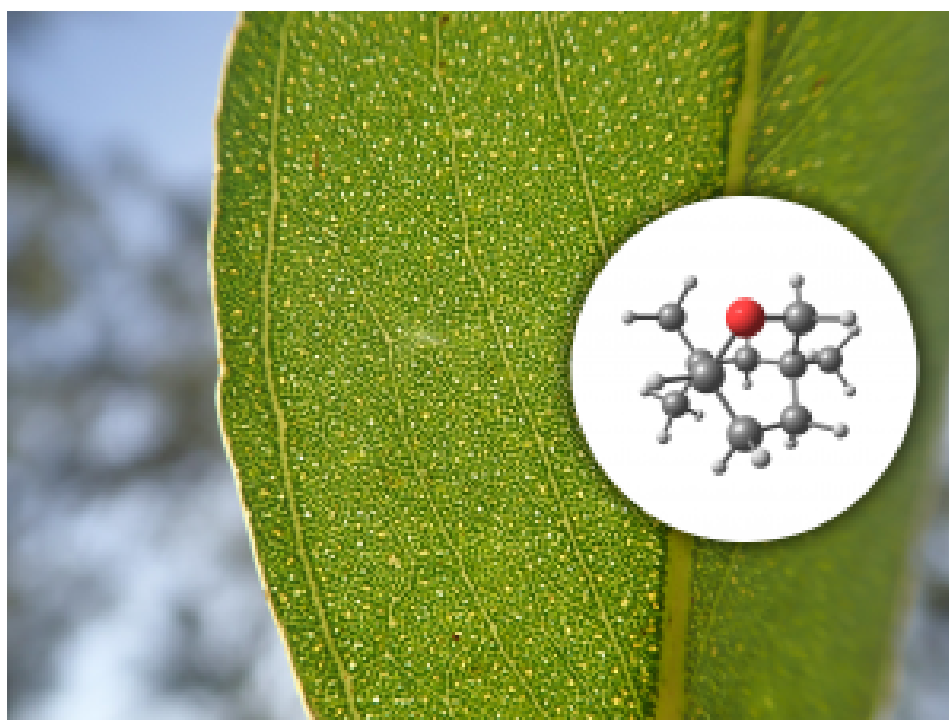
Non-antibiotic alternatives to control diseases and promote broiler growth, such as organic acids (Vieira et al., 2008), probiotics (Mountzouris et al., 2010), prebiotics (Patterson & Burkholder, 2003), and essential oils (Basmacioğlu Malayoğlu et al., 2010) have been the subject of much research in recent years.

Phytogenic solutions: proven efficacy

Essential oils, which are extracted from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, and roots, have a particularly well-established track record of medicinal applications. Efforts have centered on phytomolecules, the biologically active secondary metabolites that account for the properties of essential oils (Hernández et al., 2004; Jafari et al., 2011).

Studying these properties is challenging: essential oils are very complex natural mixtures of compounds whose chemical compositions and concentrations are variable. For example, the concentrations of the two predominant phytogenic components of thyme essential oils, thymol and carvacrol, have been reported to range from as low as 3% to 60% of the whole essential oil (Lawrence and Reynolds, 1984).

Another well-researched example is eucalyptus oil. The essential oils of eucalyptus species show antibacterial, anti-inflammatory, diaphoretic, antiseptic, analgesic effects (Cimanga et al., 2002) and antioxidant properties (Lee and Shibamoto, 2001; Damjanović Vratnica et al., 2011). The oils are mainly composed of terpenes and terpene derivatives in addition to some other non-terpene components (Edris, 2007). The principal constituent found in eucalyptus is 1,8-cineole (eucalyptol); however, other chemotypes such as α -phellandrene, p -cymene, γ -terpinene, ethanone, and spathulenol, among others, have been documented (Akin et al., 2010).



Close-up of eucalyptus leaf oil glands and the molecular structure of eucalyptol $C_{10}H_{18}O$ (red = oxygen; dark grey = carbon; light grey = hydrogen)

Antimicrobial activity

In modern intensive broiler production, bacterial diseases such as salmonellosis, colibacillosis, mycoplasmosis, or clostridia pose serious problems for the respiratory system and other areas. Analyses of the antibacterial properties of essential oils have been carried out by multiple research units (Ouwehand et al., 2010; Pilau et al., 2011; Solorzano- Santos and Miranda-Novales, 2012; Mahboubi et al., 2013; Nazzaro et al., 2013; Petrova et al., 2013).

Phenols, alcohols, ketones, and aldehydes are clearly associated with antibacterial activity; the exact mechanisms of action, however, are not yet fully understood (Nazzaro et al., 2013). Essential oils' [antimicrobial activity](#) is not attributable to a unique mechanism but instead results from a cascade of reactions involving the entire bacterial cell (Nazzaro et al., 2013). However, it is accepted that antimicrobial activity depends on the lipophilic character of the components.

The components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane-bound electron flow and thus the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP (adenosine triphosphate) pool. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Nazzaro et al., 2013; Gopi et al., 2014).

According to current knowledge, lavender, thyme, and eucalyptus oil, as well as the phytomolecules they contain, show enhanced effects when combined with other essential oils or synthetic antibiotics (Sadlon and Lamson, 2010; Bassole and Juliani, 2012; Sienkiewicz, 2012; de Rapper et al., 2013; Zengin and Baysal, 2014).

Minimum inhibitory concentration (MIC) of some essential oil components against microorganisms *in vitro*

Compounds	Microorganisms	MIC (µg/ml) or (%v/v)*	Reference
Carvacrol	<i>Bacillus subtilis</i>	0.125	Soković et al., 2010 Bajpai et al., 2012 Krishan and Narang, 2014
	<i>Candida albicans</i>	113.0–200.0	
	<i>Enterobacter cloacae</i>	0.5	
	<i>Escherichia coli</i>	0.5–225.0	
	<i>Mycobacterium avium</i>	72.0	
	<i>Pseudomonas aeruginosa</i>	1.0	
	<i>Salmonella typhimurium</i>	0.25–0.5	
1,8-cineole	<i>Staphylococcus aureus</i>	0.25–450.0	Soković et al., 2010
	<i>Bacillus subtilis</i>	4.0	
	<i>Enterobacter cloacae</i>	6.0	
	<i>Escherichia coli</i>	6.0	
Cinnamaldehyde	<i>Listeria monocytogenes</i>	5.0	Bajpai et al., 2012 Krishan and Narang, 2014
	<i>Staphylococcus aureus</i>	5.0	
	<i>Candida albicans</i>	200.0	
Eugenol	<i>Escherichia coli</i>	396.0	Bajpai et al., 2012
	<i>Salmonella sp.</i>	500	
Linalol	<i>Salmonella typhimurium</i>	0.5–16	Soković et al., 2010
	<i>Bacillus subtilis</i>	4.0	
	<i>Enterobacter cloacae</i>	6.0	
	<i>Escherichia coli</i>	6.0	
	<i>Listeria monocytogenes</i>	5.0	
Menthol	<i>Pseudomonas aeruginosa</i>	9.0	Soković et al., 2010 Mahboubi et al., 2013
	<i>Aspergillus niger</i>	125.0	
	<i>Bacillus cereus</i>	250.0	
	<i>Bacillus subtilis</i>	0.5	
	<i>Candida albicans</i>	125.0	
	<i>Enterobacter cloacae</i>	2.0	
	<i>Escherichia coli</i>	1.0–250.0	
Terpinen-4-ol	<i>Pseudomonas aeruginosa</i>	3.0	Kurekci et al., 2013
	<i>Staphylococcus aureus</i>	1.0–125.0	
Thymol	<i>Campylobacter jejuni</i>	0.05*	Soković et al., 2010 Bajpai et al., 2012 Krishan and Narang, 2014
	<i>Bacillus subtilis</i>	0.25	
	<i>Enterobacter cloacae</i>	1.0	
	<i>Escherichia coli</i>	1.0–450.0	
	<i>Pseudomonas aeruginosa</i>	1.5	
	<i>Salmonella typhimurium</i>	0.05–56.0	
	<i>Staphylococcus aureus</i>	0.25–225.0	

Immune system boost I: improved production of antibodies

Some essential oils were found to influence the avian immune system positively, since they promote the production of immunoglobulins, enhance the lymphocytic activity, and boost interferon- γ release (Awaad et al., 2010; Faramarzi et al., 2013; Gopi et al., 2014; Krishan and Narang, 2014). Placha et al. (2014) showed that the addition of 0.5g of thyme oil per kg of feed significantly increased IgA levels.

Awaad et al. (2010) experimented on birds vaccinated with the inactivated H5N2 avian influenza vaccine. The experiment revealed that adding eucalyptus and peppermint essential oils to the water at a rate of 0.25 ml per liter resulted in an enhanced cell-mediated and humoral immune response.

Saleh et al. (2014), who applied thyme and ginger oils in quantities of 100mg and 200mg per kg of feed, respectively, observed an improvement in chickens' immunological blood profile through increased antibody production. Rehman et al. (2013) stated that the use of herbal products containing eucalyptus oil and menthol in broilers showed consistently higher antibody titers against NDV (Newcastle disease virus), compared to untreated broilers.

Immune system boost II: better vaccine responses and anti-inflammatory effects

Essential oils are also used as immunomodulators during periods when birds are exposed to stress, acting protectively and regeneratively. Importantly, the oils alleviate the stress caused by vaccination (Barbour et al., 2011; Faramarzi et al., 2013; Gopi et al., 2014). The study by Kongkathip et al. (2010) confirmed the antiviral activity of turmeric essential oil.

In recent years studies have been carried out on the use of essential oils in conjunction with vaccination programs, including those against infectious bronchitis (IB), Newcastle disease, and Gumboro disease. The results of the experiments show that essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination (Awaad et al., 2010; Barbour et al., 2010; Barbour et al., 2011; Faramarzi et al., 2013).

Essential oils contain compounds that are known to possess strong anti-inflammatory properties, mainly terpenoids, and flavonoids, which suppress the metabolism of inflammatory prostaglandins (Krishan and Narang, 2014). Also, other compounds found in essential oils have anti-inflammatory, pain-relieving, or edema-reducing properties, for example, linalool from lavender oil, or 1,8-cineole, the main component of eucalyptus oil (Peana et al., 2003).

Immune system boost III: antioxidant effects and radical scavenging

An imbalance in the rate of production of free radicals or removal by the antioxidant defense mechanisms leads to a phenomenon referred to as oxidative stress. A mixture of Oregano (carvacrol, cinnamaldehyde, and capsicum oleoresin) was found to beneficially affect the intestinal microflora, absorption, digestion, weight gain and also to have an antioxidant effect on chickens (Bassett, 2000).

Zeng et al. (2015) indicated the positive effect of essential oils on the production of digestive secretions and nutrient absorption. They reduce pathogenic stress in the gut, exert antioxidant properties, and reinforce the animal's immune status.

Inside the cell, essential oils can serve as powerful scavenger preventing mutations and oxidation (Bakkali et al., 2008). Studies have demonstrated the concentration-dependent free radical scavenging ability of oils from eucalyptus species (Kaur et al., 2010; Marzoug et al., 2011; Olayinka et al., 2012). Some authors attribute the strong antioxidant capacity of essential oils to their phenolic constituents and synergistic effect between tannins, rutin, thymol, and carvacrol, and probably 1, 8-cineole. Moderate DPPH radical scavenging activity reported by Edris(2007), El-Moein et al. (2012), and Kaur et al. (2011).

Vázquez et al. (2012) have demonstrated the potential of the phenolic compounds in eucalyptus bark as a source of antioxidant compounds. The study showed that eucalyptus had ferric reducing antioxidant power in the ranges 0.91 to 2.58 g gallic acid equivalent (GAE) per 100 g oven-dried bark and 4.70 to 11.96 mmol ascorbic acid equivalent (AAE) per 100 g oven-dried bark, respectively (see also Shahwar et al., 2012). Moreover, Eyles et al. (2004) were able to show superoxide dismutase (SOD)-like activity for different compounds and fractions isolated from wood extracts.

Last but not least: positive effects on the respiratory system

In poultry production houses, especially in summer, high temperatures and low humidity increase the amount of air dust. Under such conditions, respiratory tract disorders in broiler chickens, including the deposition of particulates, become more frequent and more severe.



Clinical signs of respiratory disease in chickens include coughing, sneezing, and rales

Thyme oil, thanks to the phytochemicals thymol and carvacrol, supports the treatment of respiratory disorders. These substances smooth tightened muscles and stimulate the respiratory system. An additional advantage lies in their expectorant and spasmolytic properties (Edris, 2007).

These properties are also seen in essential oils such as eucalyptus and peppermint, which contain eucalyptol and menthol. They thin out the mucus and facilitate its removal from the airways. As a result, the airways are cleared and breathing during inflammation becomes easier (Durmik and Blache, 2012).

Another positive effect of the terpenoid compounds used in commercial preparations for poultry is that they disinfect the bronchi, preventing respiratory infections (Awaad et al., 2010; Barbour et al., 2011; Mahboubi et al., 2013). Barbour and Danker (2005) reported that the essential oils of eucalyptus and peppermint improved the homogeneity of immune responses and performance in MG/H9N2-infected broilers.

Grippozon: the phytogenic solution for respiratory health

[Grippozon](#) is a liquid composition with a high content of essential oils, which are combined to systematically prevent and ease respiratory diseases. The formulation is derived from the research on essential oils' effectiveness against respiratory pathogens that are common in animal farming. Grippozon exhibits a synergistic action of all its components to optimally support animal health. It contains a high concentration of active components; both their quantity and quality are guaranteed to deliver results.

Application of Grippozon

Grippozon application can be flexibly adapted to most common housing systems. It is fully water-soluble for use in the drinking line and it is also possible to nebulize a diluted solution in air.

The dose recommendation in drinking water usually amounts to 100ml to 200ml per 1000 liters of drinking

water (Grippozon administration has not been reported to affect water consumption). The active substances in Grippozon adhere to mouth mucosa and become volatile in the breathing air later on. Therefore Grippozon can enter the respiratory system indirectly as well. The volatile compounds also spread into the whole barn air and, thus, indirectly via breathing into the respiratory system (and farmers notice the smell of essential oils when Grippozon is applied through in the waterline)

Grippozon can also be used as a spray at a rate of 200ml/10 liters of water for 2000 birds, twice daily on 2-3 days a week. This produces a very effective nebulization effect and offers faster respiratory relief to birds.

Grippozon is an impactful tool for managing respiratory problems. Thanks to its effective mucolytic and relaxant activity, Grippozon gives symptomatic relief to the birds during high-stress periods of respiratory diseases. Mucus in the trachea works as media for the proliferation of bacteria and viruses, so by thinning the mucus, Grippozon slows down the proliferation of bacteria and the spread of disease. Grippozon helps in improving air quality and air intake. It can also be used to stimulate the immune response during vaccination.

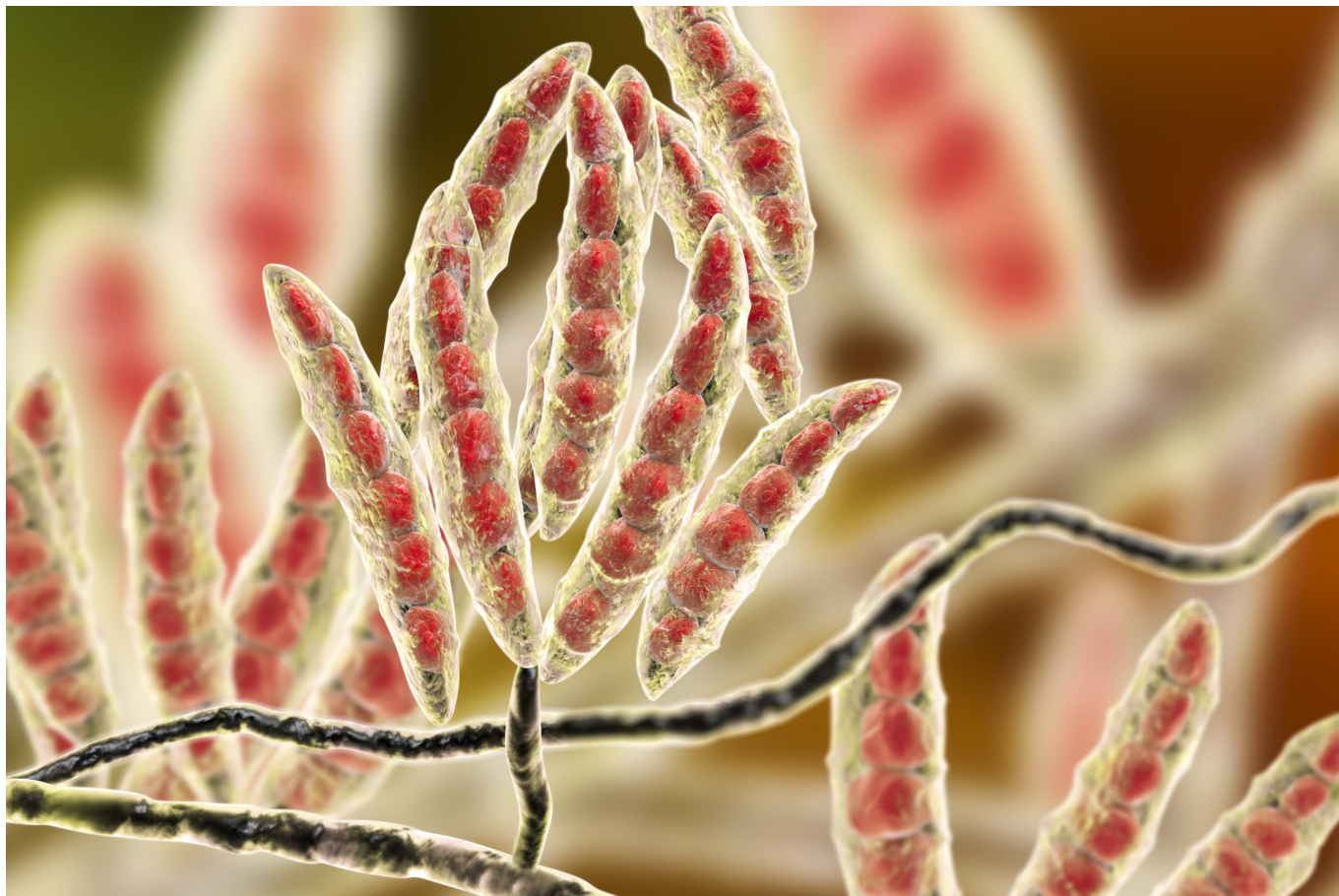
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References available on request

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

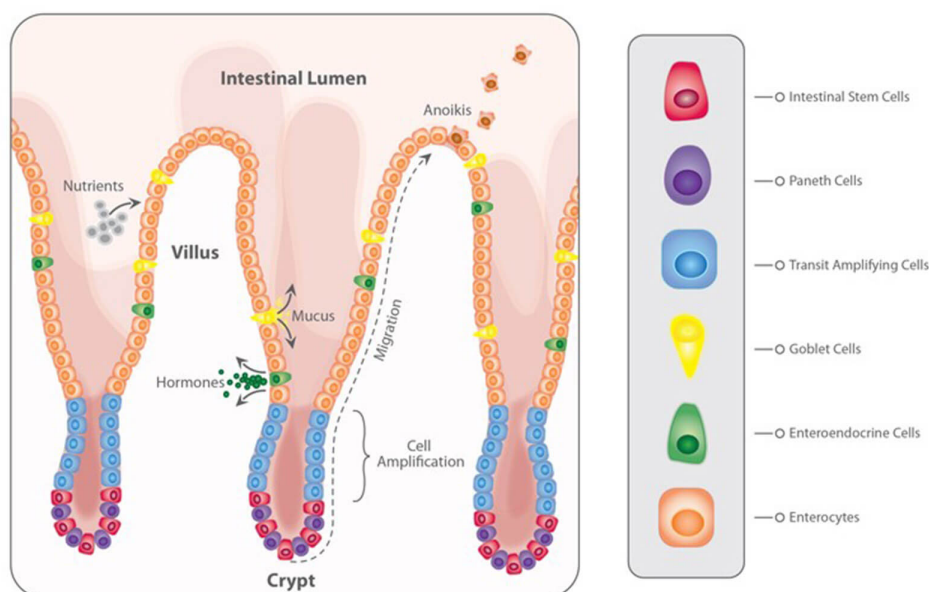
When mycotoxins are ingested, they encounter the GIT's intestinal epithelium (Figure 1). This single layer of cells lining the intestinal lumen serves two conflicting functions: firstly, it must be permeable enough to allow the absorption of nutrients. On the other hand, it constitutes the primary physiological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

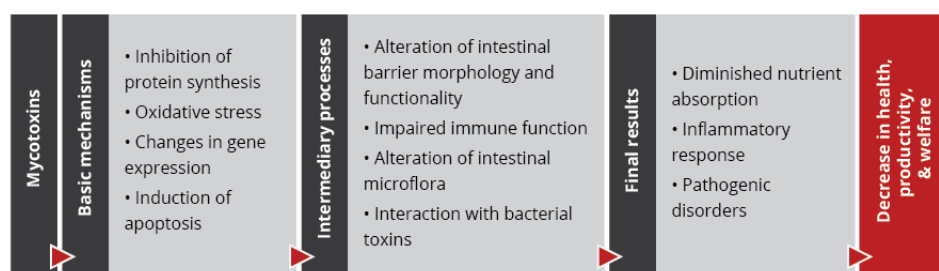
Adapted from: [Biehl et al., 1993](#); [Bouhet & Oswald, 2007](#); [Devreese et al., 2015](#); [Ringot et al., 2006](#)

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

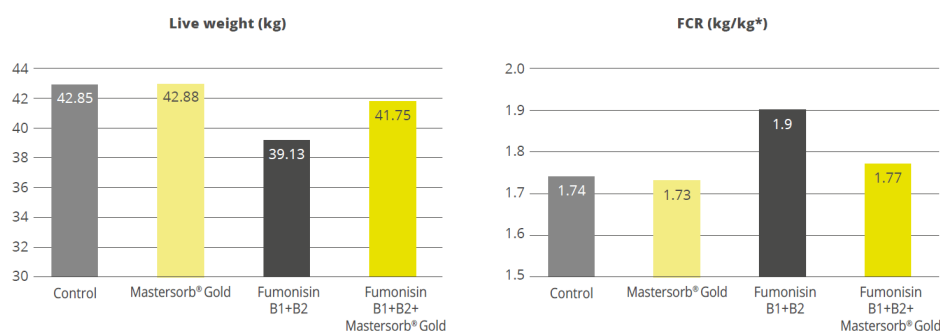
To prevent the detrimental consequences of mycotoxins on animal health and performance, proactive solutions are needed that support the intestinal epithelium's digestive and immune functionality and help maintain a balanced microbiome in the GIT. Moreover, it is crucial for any anti-mycotoxin product 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

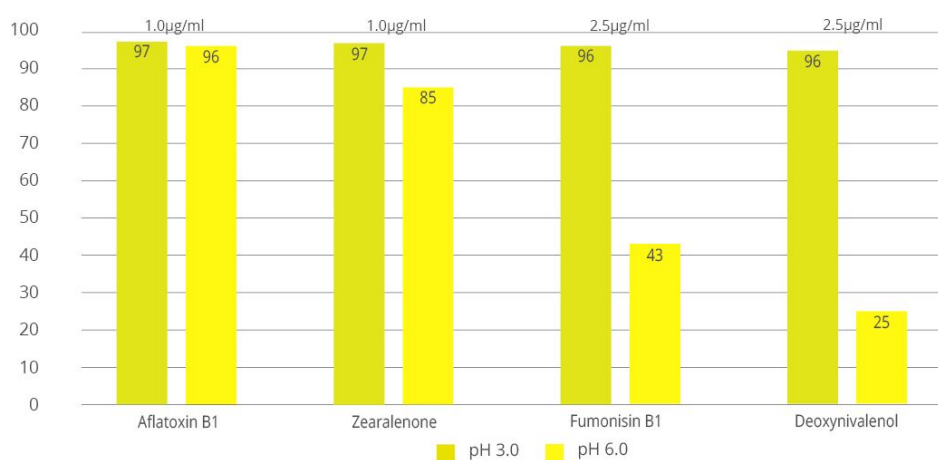


Another study of crossbred female piglets, carried out at a German university, investigated whether Mastersorb Gold could support performance as well as liver health under a naturally occurring challenge of ZEA (~ 370ppb) and DON (~ 5000ppb). Mastersorb Gold significantly improved weight gain and feed 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 Technical Team, EW Nutrition

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.

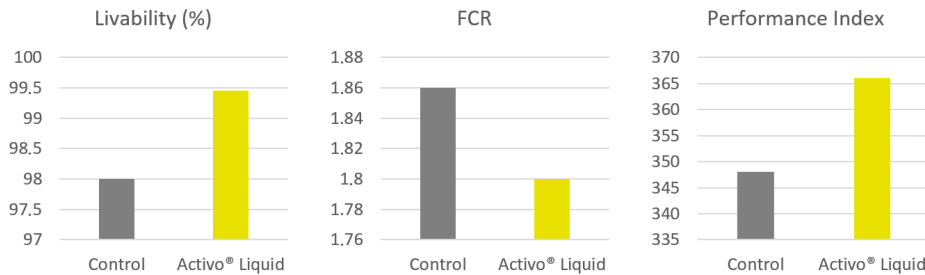
The effect of phytomolecules on broilers during a challenging phase

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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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.

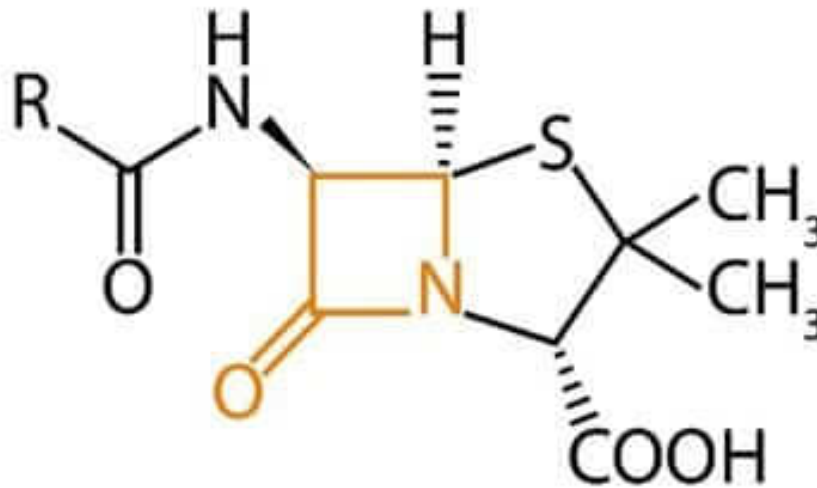
The excessive use of antibiotics, however, leads to the [occurrence of antimicrobial resistance](#) (AMR): due to mutations, resistance genes are created which enable enterobacteria such as *Salmonella*, *Klebsiella* and *E. coli* to produce enzymes (β -lactamases) in order to withstand β -lactam antibiotics. 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 β -lactam antibiotics work

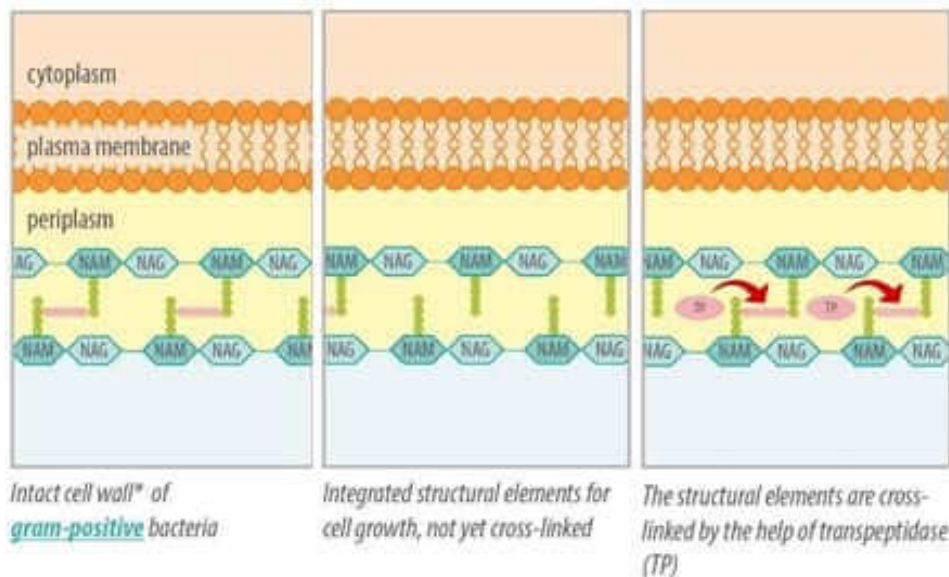
The group of β -lactam antibiotics consists of penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics are characterised by their lactam ring (Figure 1).

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



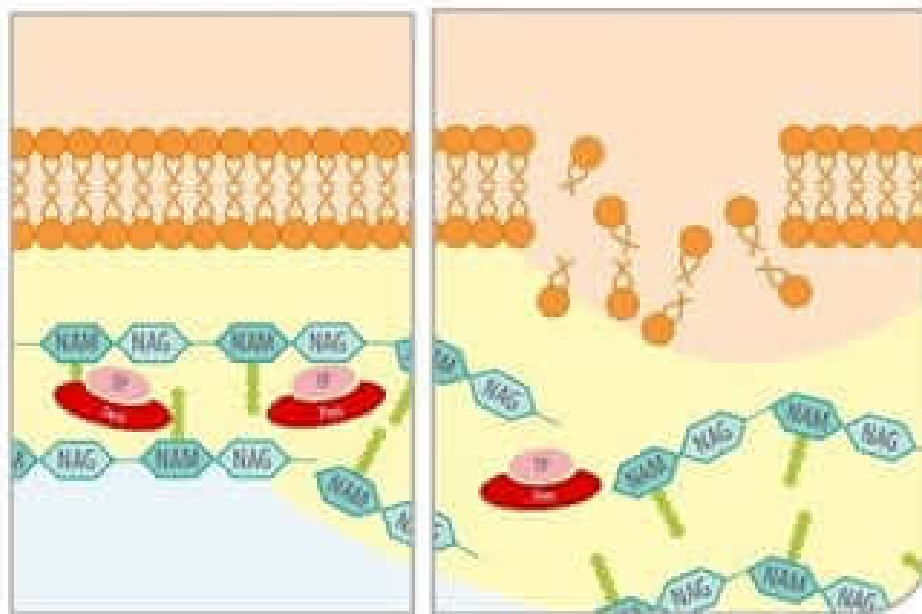
If bacteria are growing, the cell wall also has to grow. For this purpose existing conjunctions are cracked and new components are inserted. In order for the cell wall to remain a solid barrier, the new components must be interconnected by crosslinks. For the creation of these crosslinks an enzyme is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

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



Due to their structure, β -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 β -lactam antibiotics act bactericidal.

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



If the transpeptidase is blocked e.g. by Penicillin (Pen), cross-linking is not possible

The unstable cell wall does not withstand the pressure any more, the bacteria cell gets destroyed

The challenge: *E. coli* producing β -lactamases

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

Another mutation leads to so-called AmpC (aminopenicillin and cephalosporin) β -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

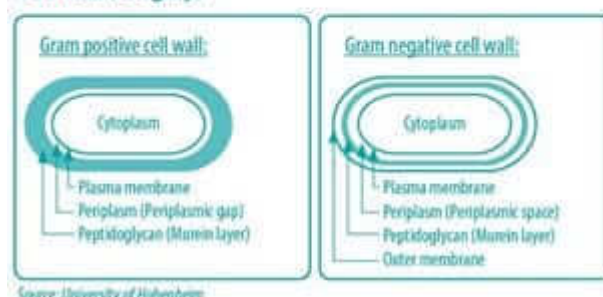
(Adenosin-tri-phosphate). It leads to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

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).

Gram-positive bacteria and gram-negative bacteria:
Bacteria differ in the construction of their cell walls. The Danish bacteriologist Hans Christian Gram (1853–1938) developed a colouring method to differentiate the bacteria. It is not possible to assign all bacteria to both groups. There are also gram-variable and gram-indefinite species.

Cell wall (roughly):



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 served 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, Scotland	Cefotaxime	Phytomolecules (Activo® Liquid)			
	30 µg / ml	0.1 %	0.2 %	0.4 %	0.5 %
<i>E. coli</i> ESBL 1 (Poultry)	-	+	++	++	++
<i>E. coli</i> ESBL 2 (Poultry)	-	+	++	++	++
<i>E. coli</i> AmpC 1 (Poultry)	-	+	++	++	++
<i>E. coli</i> AmpC 2 (Poultry)	-	++	++	++	++
<i>E. coli</i> 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 %
<i>E. coli</i> ATCC25922	+	++	++	++
<i>E. coli</i> ESBL 1 (Pig)	-	++	++	++
<i>E. coli</i> ESBL 2 (Pig)	+	++	++	++
<i>E. coli</i> ESBL 3 (Poultry)	+	++	++	++
<i>E. coli</i> 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:

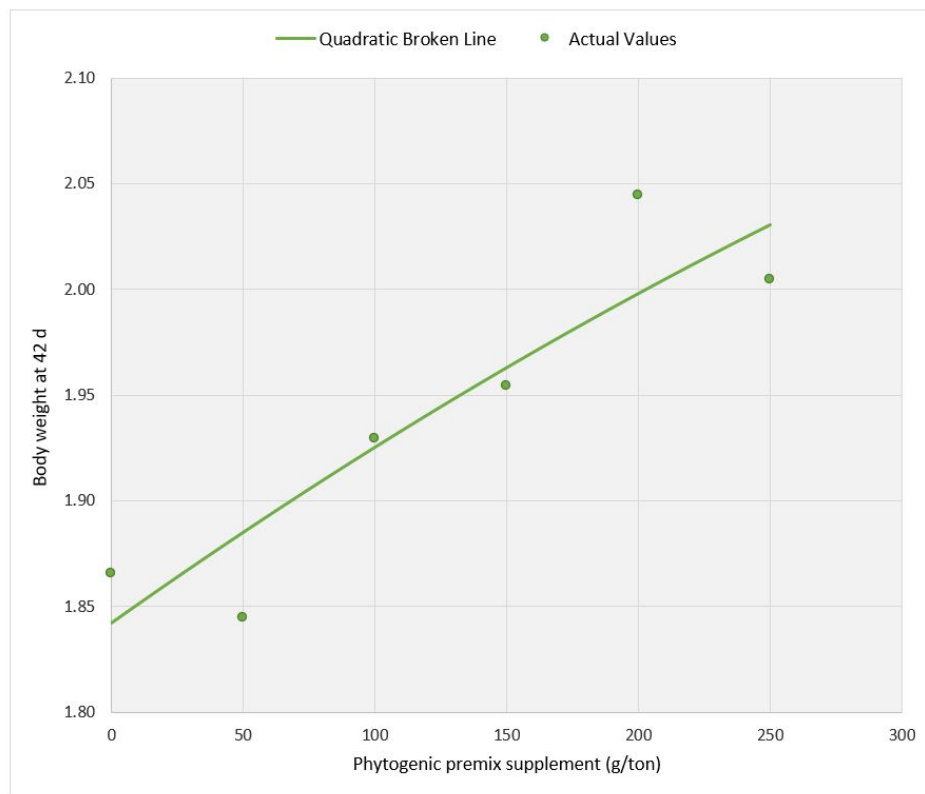
- No supplement (negative control)
- 50 g of Activo /US ton of feed
- 100 g of Activo /US ton of feed
- 150 g of Activo /US ton of feed
- 200 g of Activo /US ton of feed
- 250 g of Activo /US ton of feed
- Antibiotic growth promoter (AGP)(positive control)

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 phytogetic premix added to their diet the better feed efficiency (Figure 3).

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

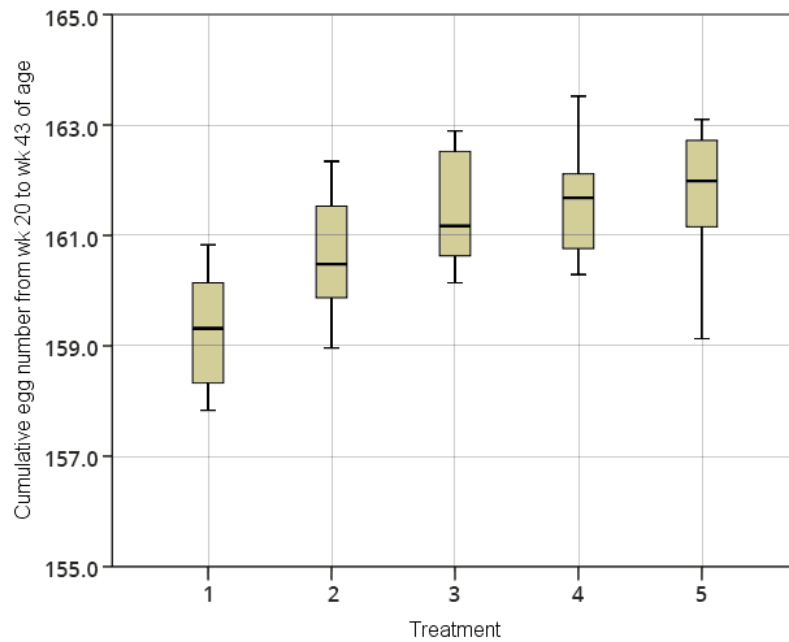
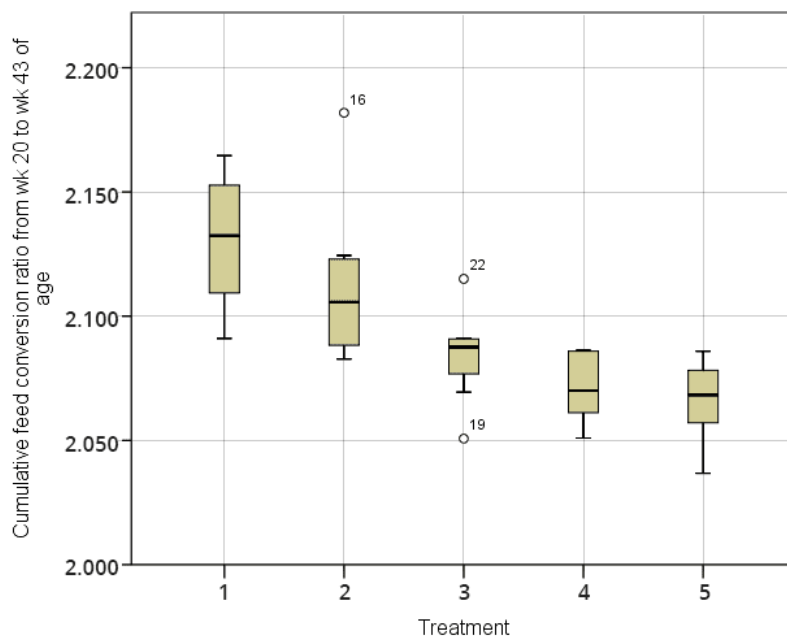


Figure 3: Dose-dependent effects of Activo on feed efficiency in laying hens



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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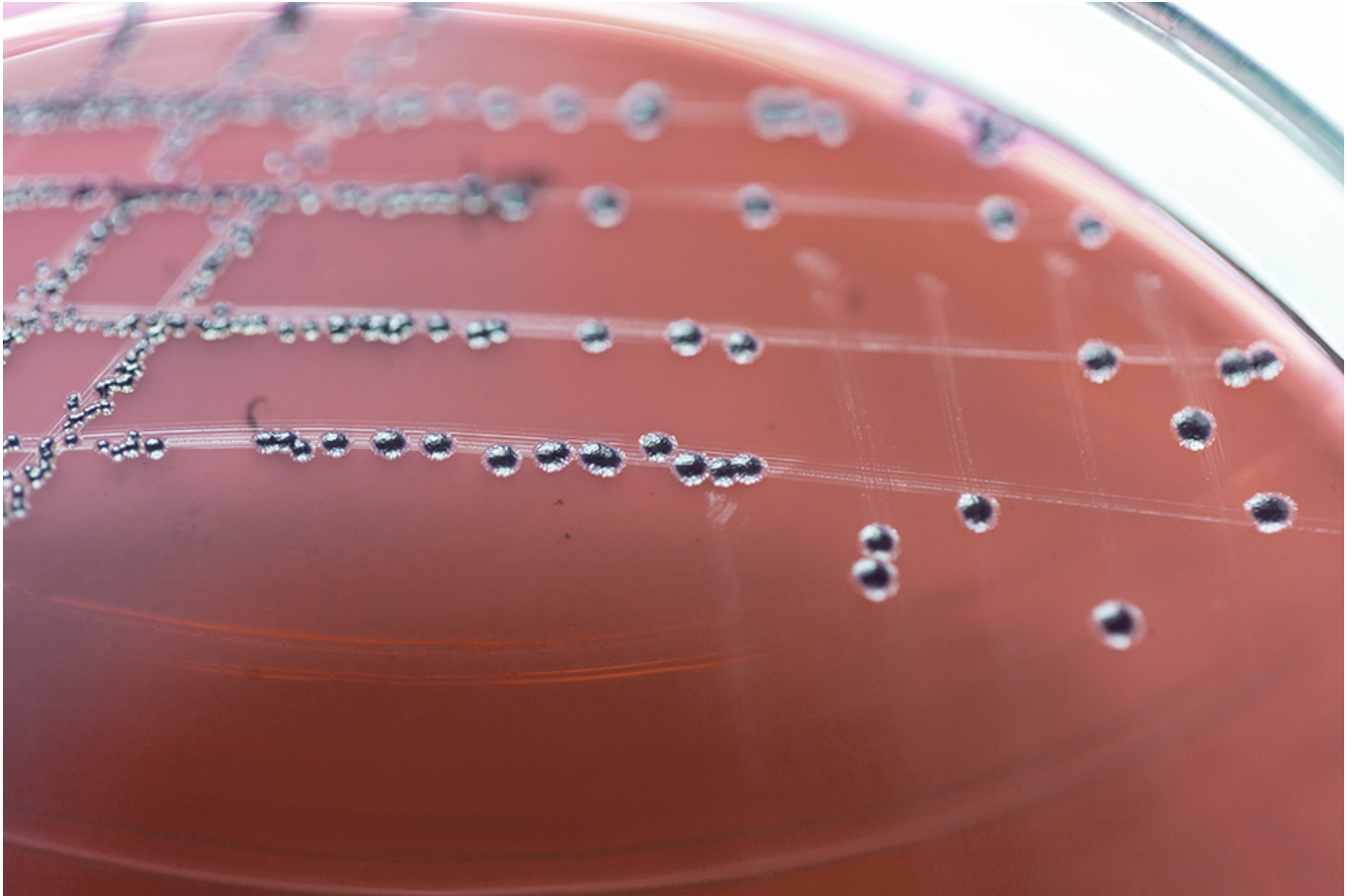
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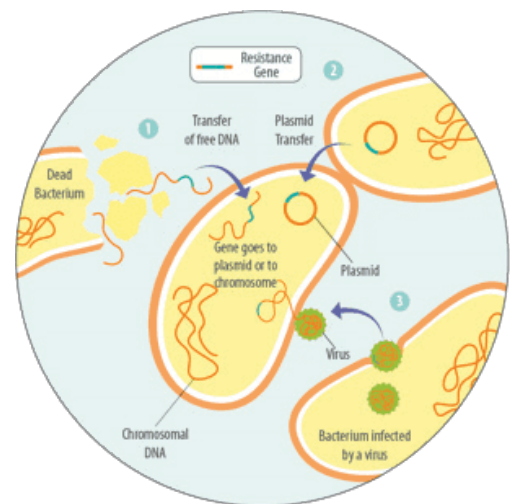
Photo source: [Aviagen](#)

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

	Activo Liquid		
Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)	10%	2%	1%
<i>E. coli</i> (reference strains)	++	+	+
<i>Proteus vulgaris</i> (reference strains)	+	+	+
<i>Pseudomonas fluorescens</i>	++	+	-
<i>Salmonella pulmorum</i>	++	++	+
<i>Salmonella gallinarum</i>	++	++	+
<i>Staphylococcus aureus</i> (reference strains)	+++	++	++
- no effect + growth <u>inhibiting</u> ++ bactericidal			

Table 1: Effect of Activo Liquid against standard pathogens

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*, Enter- 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.

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

	Activo Liquid			
Laboratory: <u>Vaxxinova</u> , Münster, Germany	0.1%	0.2%	0.4%	1%
<u>E.coli</u> Reference ATCC25922	+	++	++	++
ESBL 1 (Pig)	-	++	++	++
ESBL 2 (Pig)	±	++	++	++
ESBL 3 (Poultry)	±	++	++	++
ESBL 4 (Poultry)	-	++	++	++
<u>S. aureus</u> <u>Referenz</u> ATCC29213	-	+	++	++
MRSA 1 (Pig)	-	+	++	++
MRSA 2 (Pig)	-	+	+	++
- <u>no effect</u> + <u>growth inhibiting</u> <u>± bacteriostatic</u> ++ <u>bactericidal</u>				

Table 2: Effect of Activo Liquid against field-isolated standard pathogens

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.

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.

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.