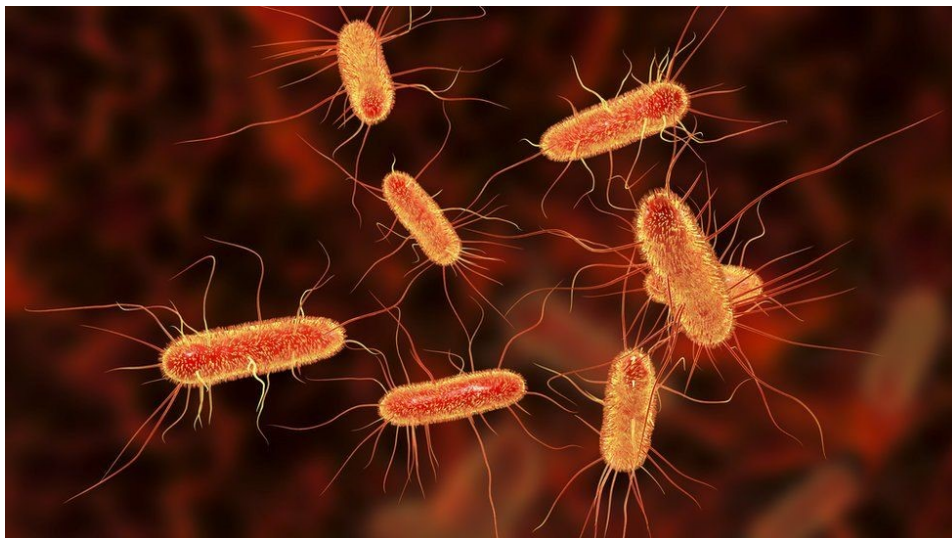


# The hidden danger of endotoxins in animal production



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).

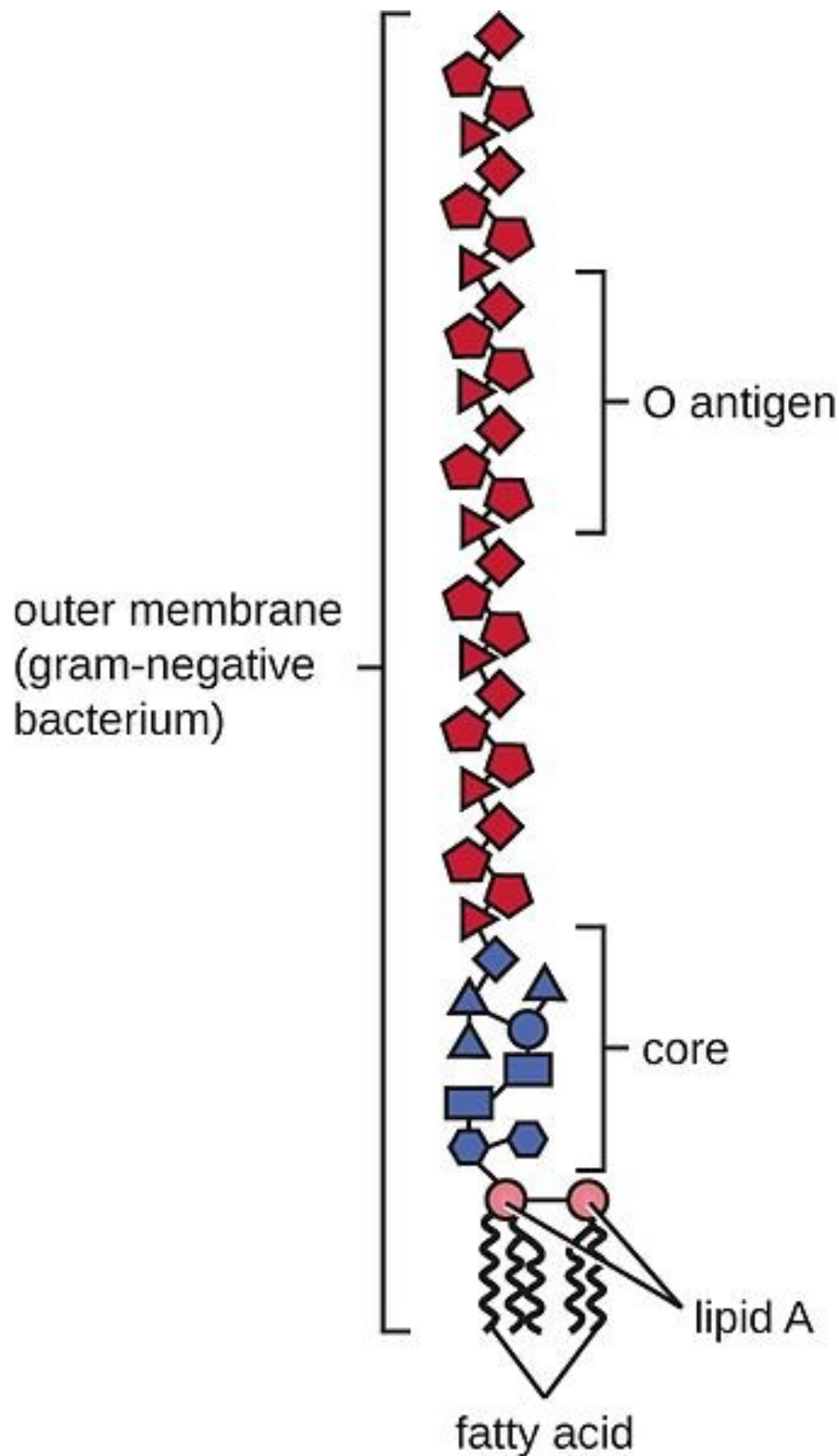


Figure 1: Structure of an LPS

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 $\beta$ , IL-6, IL-8, and tumor necrotic factor (TNF)  $\alpha$  and  $\beta$ . 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).

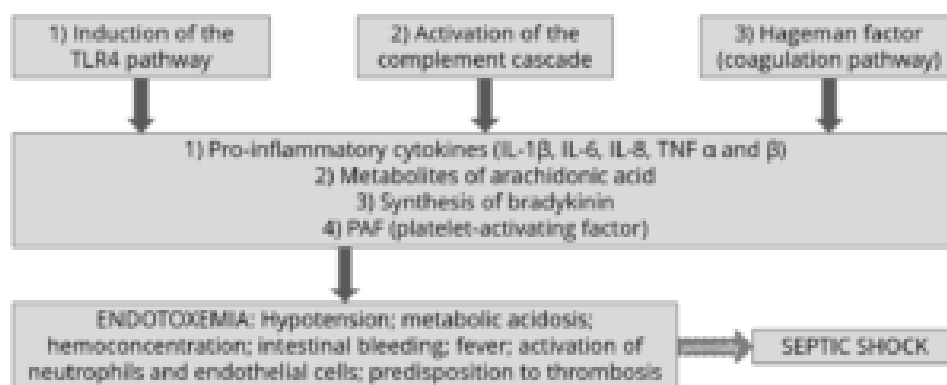


Figure 2: How LPS leads to endotoxemia – 3 modes of action

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  $\alpha$  and  $\beta$ , IL-6 or IL-8, and fibrin and fibrinogen (though they are not specific to endotoxemia). It is vital to carry out a blood sample analysis to deduce a possible endotoxemia from symptoms and performance losses in the animal.

## How the metabolic effects of endotoxemia depress performance

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



to continue; hence, the energy required for producing heat will be diverted from the energy usually spent on producing milk, egg, meat, etc., and performance suffers.

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

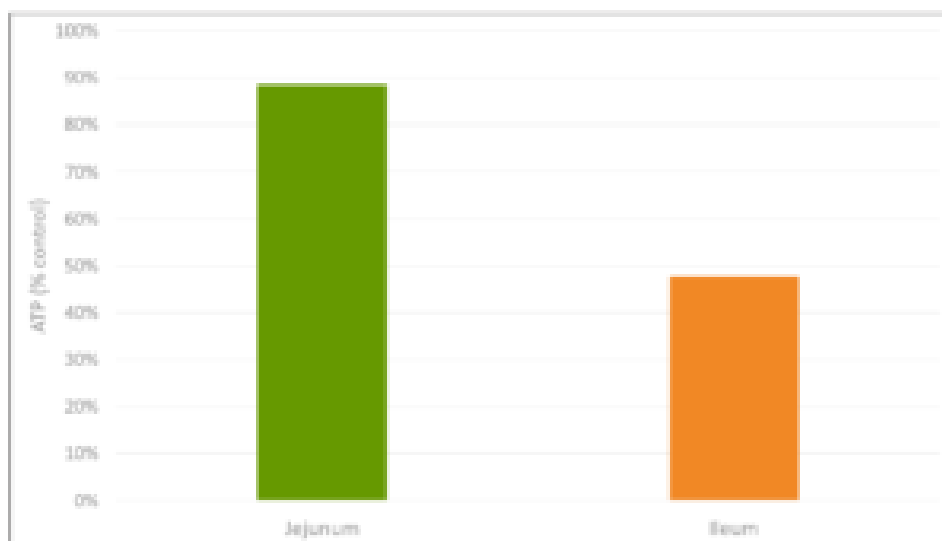


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from [Li et al., 2015](#))

A [piglet study by Huntley, Nyachoti, and Patience \(2017\)](#) took this idea further (Figure 4): 3 groups of 10 Yorkshire x Landrace pigs, weighing between 11 and 25 kg, were studied in metabolic cages and in respiratory chambers. This methodology allows for simultaneous measurement of oxygen consumption, CO<sub>2</sub> 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.

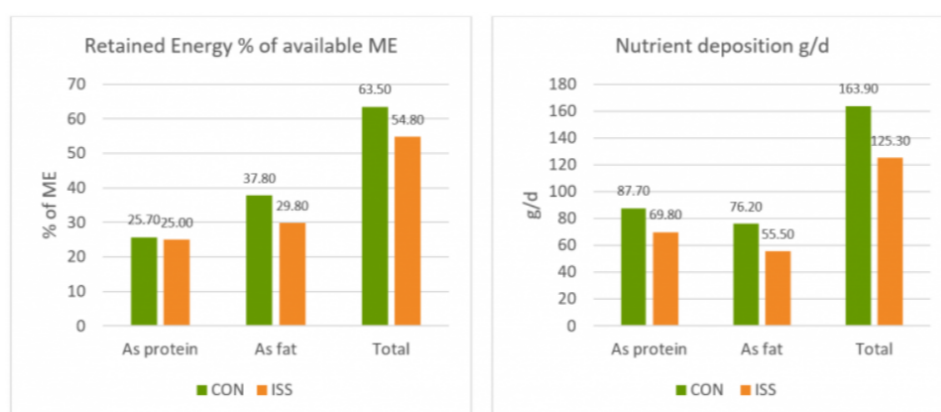


Figure 4: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from [Huntley, Nyachoti, and Patience, 2017](#))

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.

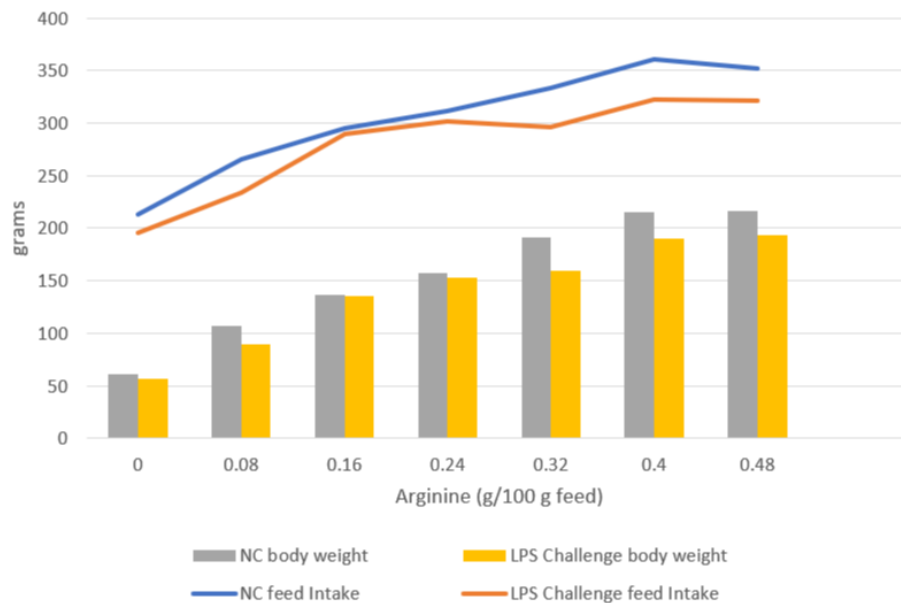


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on [Webel, Johnson, and Baker, 1998](#))

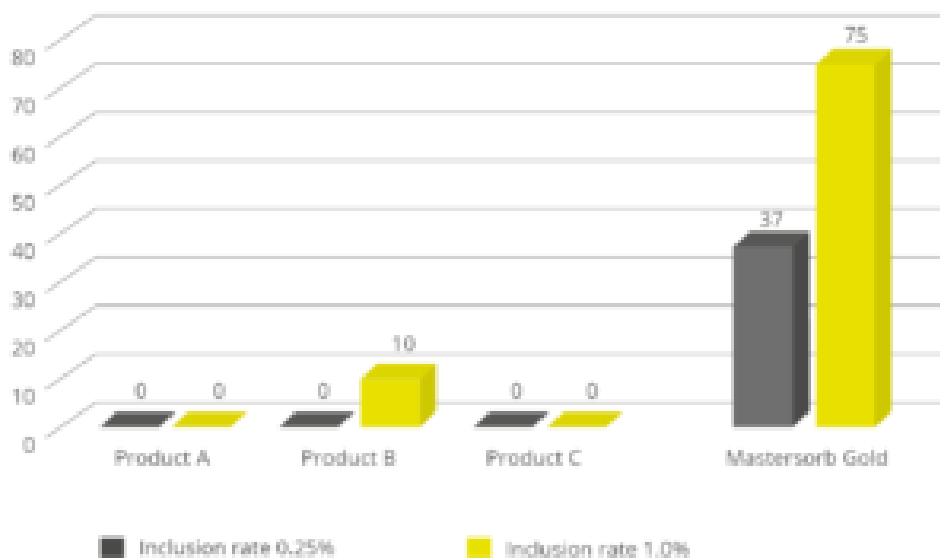
NC = negative control

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 phytochemicals 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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# Mycotoxin interactions: An obstacle to risk assessment



**In animal feed, multi-mycotoxin contamination is found quite frequently and seems to be the rule rather than the exception in practical diets. Here is a quick overview of the known interactions.**

## What are the most common mycotoxins in feed?

Mycotoxins represent an exceptional challenge for feed and animal producers: they are produced by common molds, occur in a great variety and number, are sporadic or heterogeneous in their distribution, and their effects on farm animals are seldom recognized as mycotoxicosis. Among hundreds of known mycotoxins, aflatoxins, mainly produced by *Aspergillus* species, ochratoxin A, produced by *Aspergillus* and *Penicillium* species, as well as fumonisins, trichothecenes (especially DON and T-2 toxin) and zearalenone, primarily produced by many *Fusarium* species stand out as the most common contaminants.

## Consequences of mycotoxin contamination

Ingestion of these mycotoxins may cause an acute toxicity or chronic disorders, depending on the concentration and duration of exposure. In farm animals, this might manifest as decreased performance, feed refusal, poor feed conversion, reduced body weight gain, immune suppression, reproductive disorders, and residues in animal food products.

Due to their frequent occurrence and their severe toxic properties, many countries appointed legal regulations or guidance for the major mycotoxins to protect animals and human consumers. The current regulations are typically very specific in terms of animal species and even for the production stage considering that mycotoxins affect for example poultry in a different way than cattle and broilers in a different way than breeders or laying hens. The threshold and/or guidance values for each species, however, were determined based on toxicological data from studies investigating a monoexposure leaving out the possibility of any combined effects of mycotoxins.

# Multi-contamination: the rule, not the exception

If we were able to ensure that the animals were exposed to only one mycotoxin at a time, following the regulatory guidelines would allow us to protect our animals in most of the cases. Several worldwide surveys show, however, that mycotoxin multicontamination of animal feed is found very frequently\* and seems to be the rule rather than the exception in practical diets. The concurrent appearance of mycotoxins in feed can be explained as follows: each mold species has the capacity to produce a number of mycotoxins simultaneously. Each species, in turn, may infest several raw materials leaving behind one or more toxic residue. In the end, a complete diet is made up of various raw materials with individual mycotoxin loads resulting in a multitude of toxic challenges for the animals.

Several researchers showed that the effects observed during multiple mycotoxin exposure can differ greatly from the effects observed in animals exposed to a single mycotoxin, indicating that the simultaneous presence of mycotoxins may be more toxic than predicted from the mycotoxins alone. This is because mycotoxins interact with each other. The interactions can be classified into three main different categories: *antagonistic*, *additive*, and *synergistic*.

## Types of mycotoxin interactions

**Additivity** occurs when the effect of the combination equals the expected sum of the individual effects of the two toxins (*Figure 1a*).

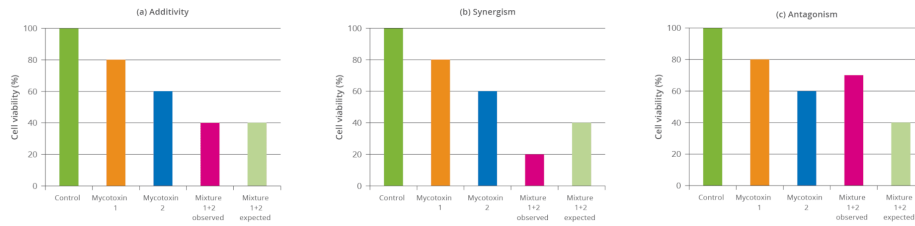
**Synergistic** interactions of two mycotoxins lead to a greater effect of the mycotoxin combination than would be expected from the sum of their individual effects (*Figure 1b*). A special form of synergy, sometimes called potentiation, occurs when one or both of the mycotoxins do not induce effects whereas the combination induces a significant effect.

When the effect of the mycotoxin combination is lower than expected from the sum of their individual effects, **antagonism** can be observed (*Figure 1c*). In general, most of the mycotoxin mixtures lead to additive or synergistic effects, highlighting a significant threat to animal health and being the major reason that impedes risk assessment. Synergistic actions may occur when the single mycotoxins of a mixture act at different stages of the same mechanism, e.g. T-2 increases ROS production while AFB1 decreases its clearance when the presence of one mycotoxin increases the absorption of another or decreases its metabolic degradation.

## Be aware of contaminations

Given their complex interactions, the toxicity of [combinations of mycotoxins](#) cannot merely be predicted based upon their individual toxicities. Knowing that even low levels of mycotoxin combinations can harm animal productivity, health, and welfare, it is useful for feed and animal producers to be aware of present contaminations, to be able to [link them to the risk they pose](#) for the animal and consequently take actions before the problems appear in the field.

Figure 1 - Characterisation of the interaction between mycotoxins (Smith et al., 2016).



\*References are available on request.

**By Technical Team, EW Nutrition**

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# Using milk thistle to reduce liver damage from mycotoxins



**Mycotoxins not only reduce animal performance, but they also cause significant liver damage.**

**The seeds of the herb plant milk thistle contain a mixture of flavonolignans known as [silymarin](#) and can help in [reducing liver damage](#)**

## **when animals get in contact with mycotoxin contaminated feed.**

Mycotoxins are a constant problem in cereals causing economic losses to the global animal industry. Mycotoxins are produced by filamentous fungi varying widely in their chemical and biological characteristics and effects on animals. Among the various mycotoxins, aflatoxins, and more specifically aflatoxin B1, is one of the most problematic because it affects maize, one of the major staple ingredients in animal diets worldwide. Of course, in nature, mycotoxins mostly occur in combinations, but even with singly contaminated ingredients, the nature of animal feeds leads to the concurrent presence of multiple mycotoxins, coming from the different ingredients. The separation of mycotoxins in polar and non-polar, however, simplifies their management. For example, aflatoxins (polar) are easily addressed by the inclusion of an adsorbent (like bentonite, for example). The same ingredient adsorbs not only aflatoxins, but also other mycotoxins, like zearalenone, ochratoxin A, and T-2 toxin, albeit at reduced efficiency.

### **Products limited to work in gut**

Certainly, anti-mycotoxin agents are effective only while the feed is being digested, that is, while the feed remains in the lumen of the [gastrointestinal tract](#). Anti-mycotoxin agents are not absorbed by the animal, whereas non-adsorbed mycotoxins are; leading to the need for further detoxification within the organism. Parts of mycotoxins might enter the organism despite the use of an anti-mycotoxin agent in feed due to the fact that no product is 100% effective, not all mycotoxins are affected similarly by a single product, non-polar mycotoxins might not be inactivated if only a polar agent is used, and vice versa and lastly, high contamination might render the normal dosage inadequate. This is often seen as being the most common cause, In other words, part of mycotoxins in the feed can still enter the animal. The exact effects on animal health and performance will depend, of course, on the initial contamination levels in the feed and on the constitution of the liver.

### **Mycotoxins and liver damage**

Even short-term exposure to mycotoxins suffices to cause significant liver damage and loss of performance. In a study (Meissonnier, 2007), pigs were given 385, 867, or 1807 µg aflatoxin B1/kg feed for four weeks. Pigs receiving the highest level of aflatoxin developed clear signs of aflatoxicosis: hepatic dysfunction and decrease in weight gain. Also, the pigs exposed to the lower levels of mycotoxins showed clear signs of impaired metabolism and biotransformation. Additionally, mycotoxins and particularly aflatoxins inhibit the major hepatic biotransformation enzymes. This has significant consequences in veterinary medication applications as animals become unable to clear medications from their system – and of course, other toxins.

Read [Using milk thistle to reduce liver damage from mycotoxins](#) the full article  
*ALL ABOUT FEED, Volume 23, No. 3, 2015*