Decoding the connection between stress, endotoxins, and poultry health

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

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

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

What are endotoxins?

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

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

The gut is critically affected by stress

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

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

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

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

Technological stress

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

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

Nutritional stress

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

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

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

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

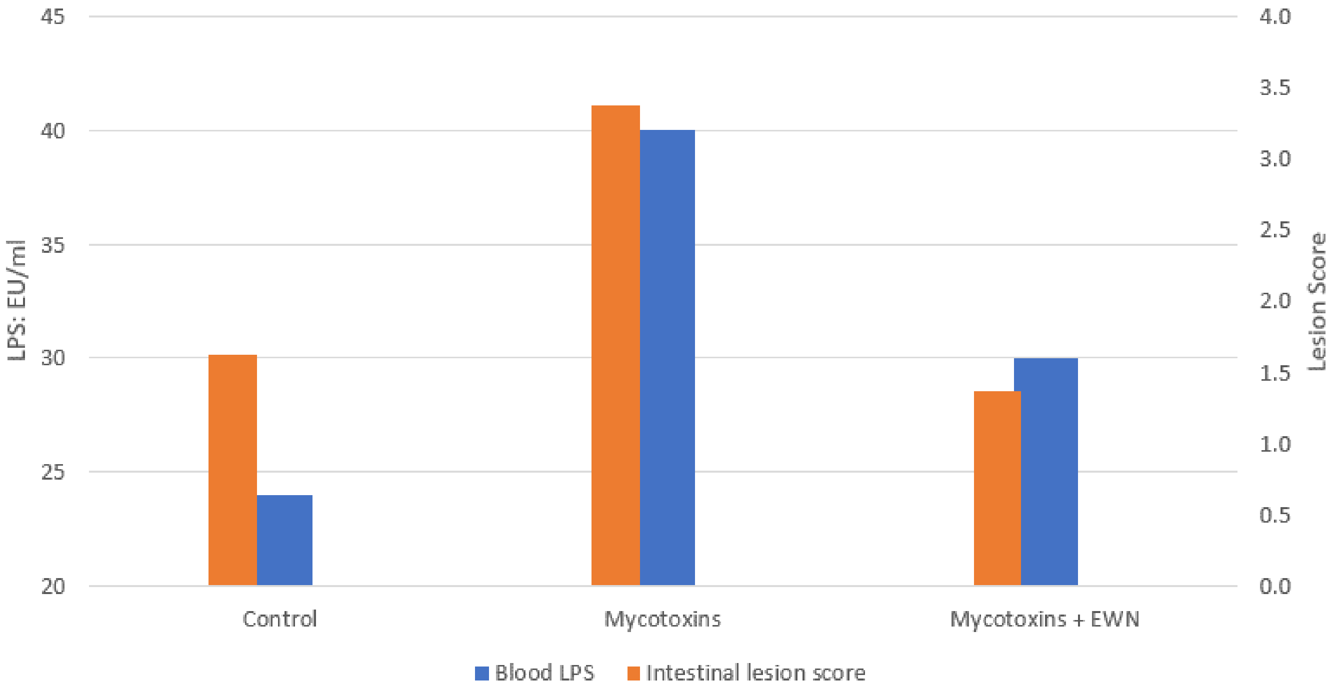


Figure 1 Blood LPS and intestinal lesion score of broilers challenged with 200ppb AFB1 + 350 ppb OTA from 1 to 21 days of age without and with an anti-toxin product from EW Nutrition GmbH (adapted from Caballero et al., 2021)

Pathogenic stress

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

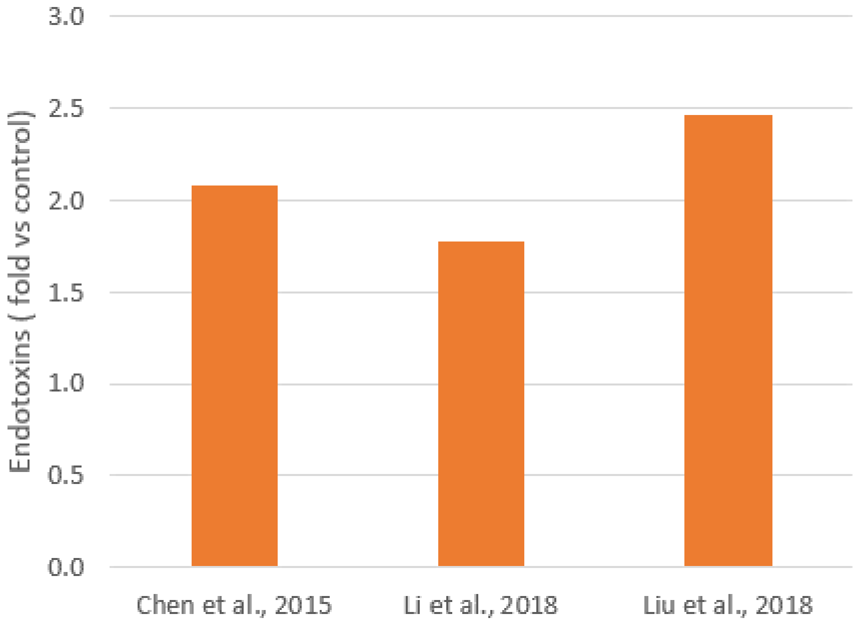


Figure 2 Increase in systemic LPS (vs a healthy control) after a NE challenge (adapted from Chen et al., 2015, Li et al., 2018 & Liu at al., 2018)

Environmental stress

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

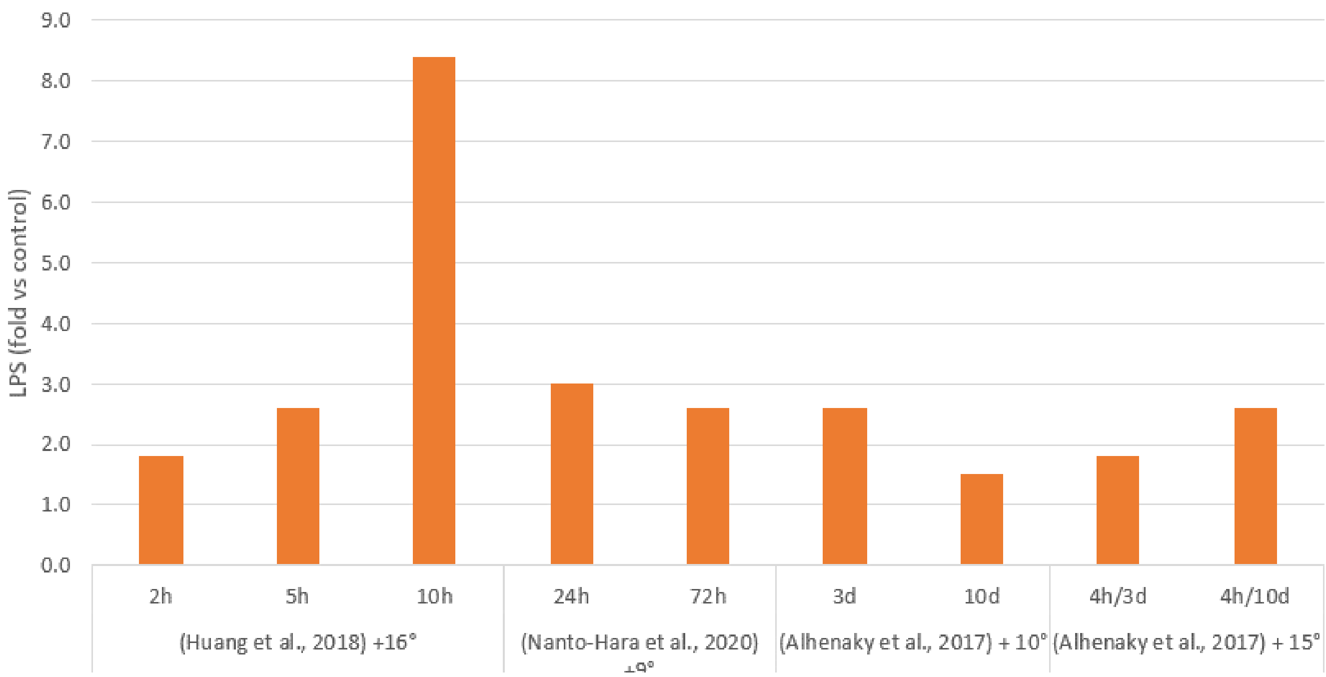
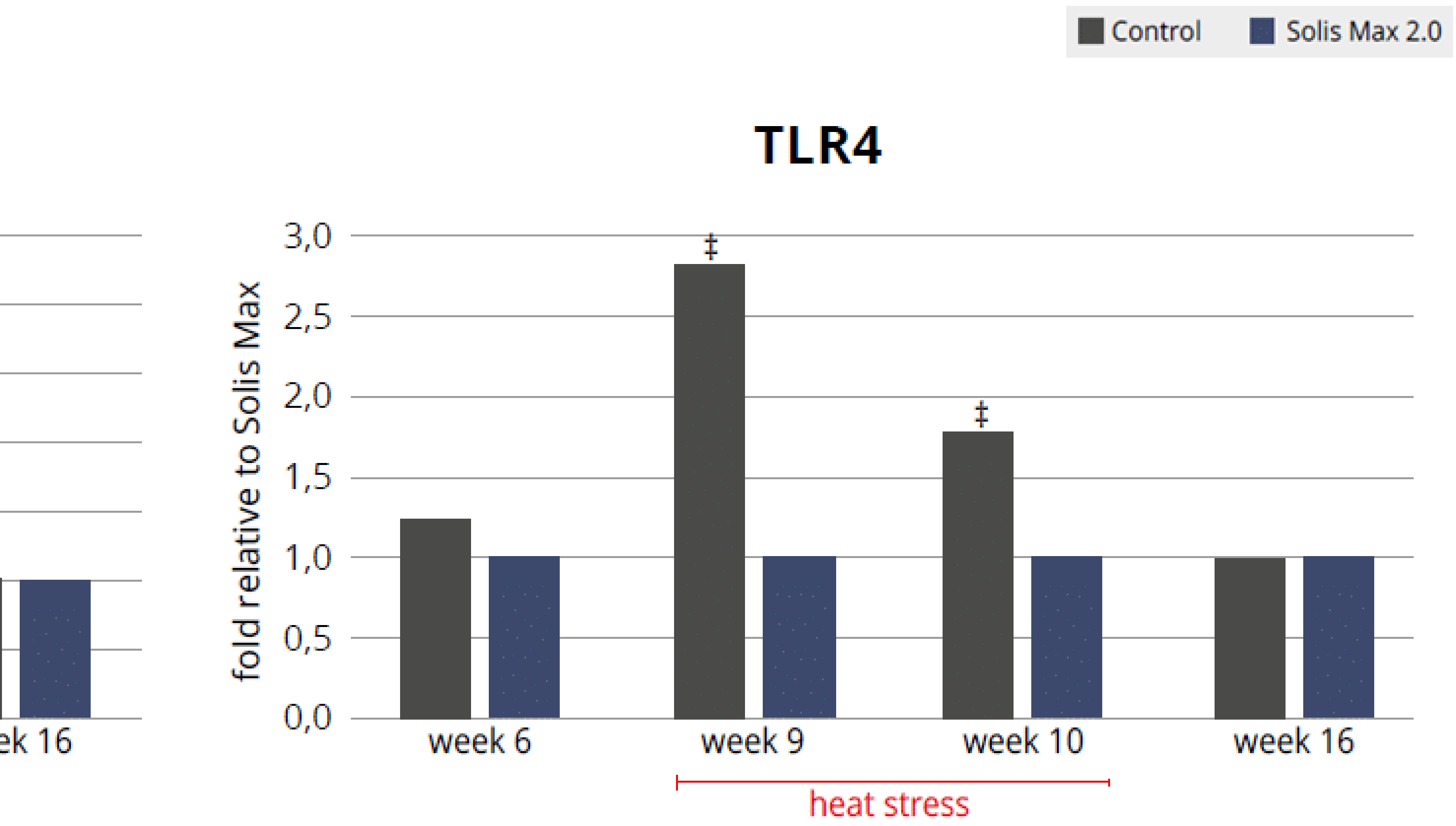


Figure 3 Systemic LPS increase (in comparison with a non-stressed control) after different heat stress challenges in broilers: 16°C increased for 2, 5 and 10 hours (Huang et al., 2018); 9°C increased for 24 and 72 hours (Nanto-Hara et al., 2020); 10°C continuously for 3 and 10 days, and 15°C 4 hours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

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

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

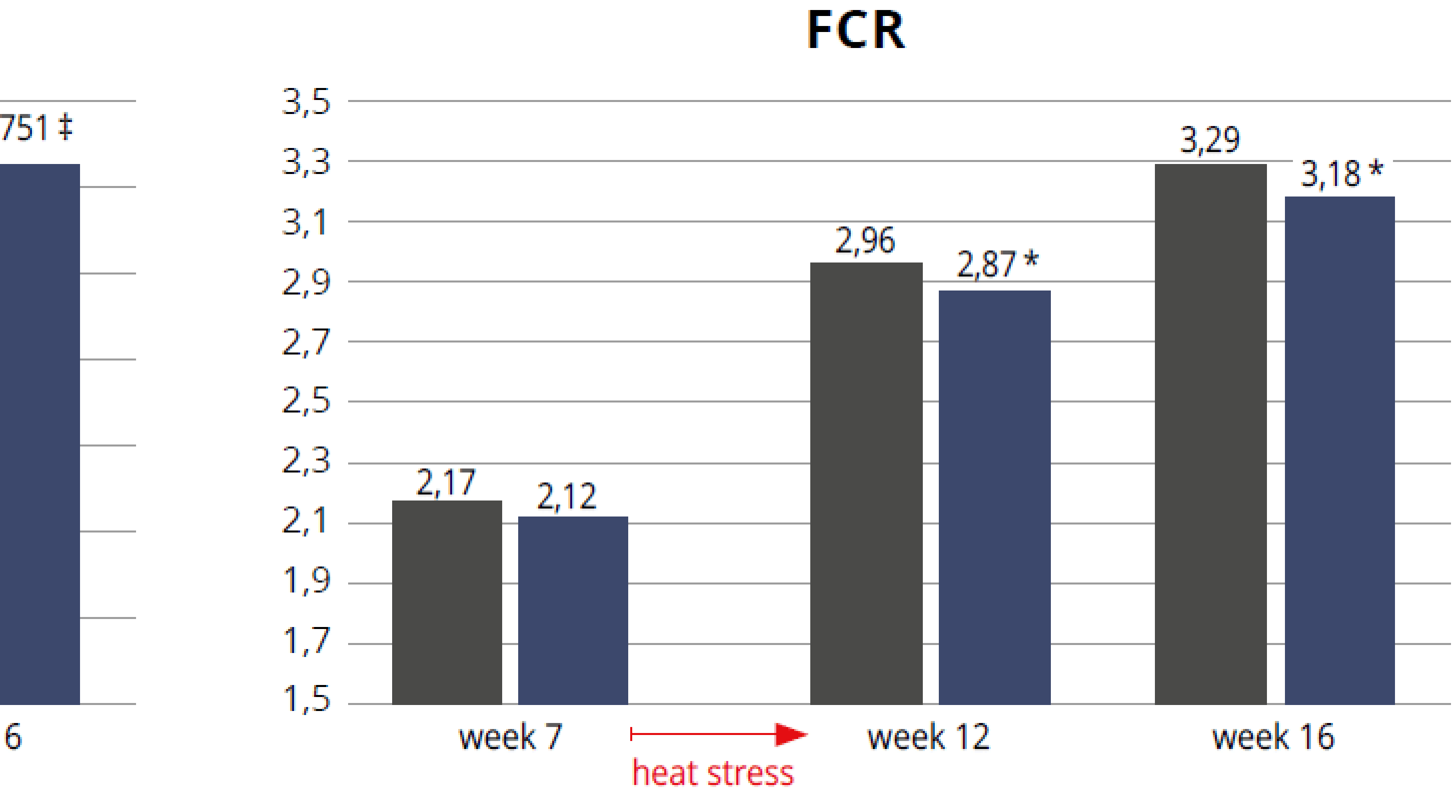
Throughout the heat stress period, blood LPS (Fig 4) was lower in the pullets receiving the product, which allowed lower inflammation, as evidenced by the lower expression of TLR4 (Fig. 5). Oxidative stress was also mitigated with the help of the combination of phytomolecules in the product, obtaining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



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

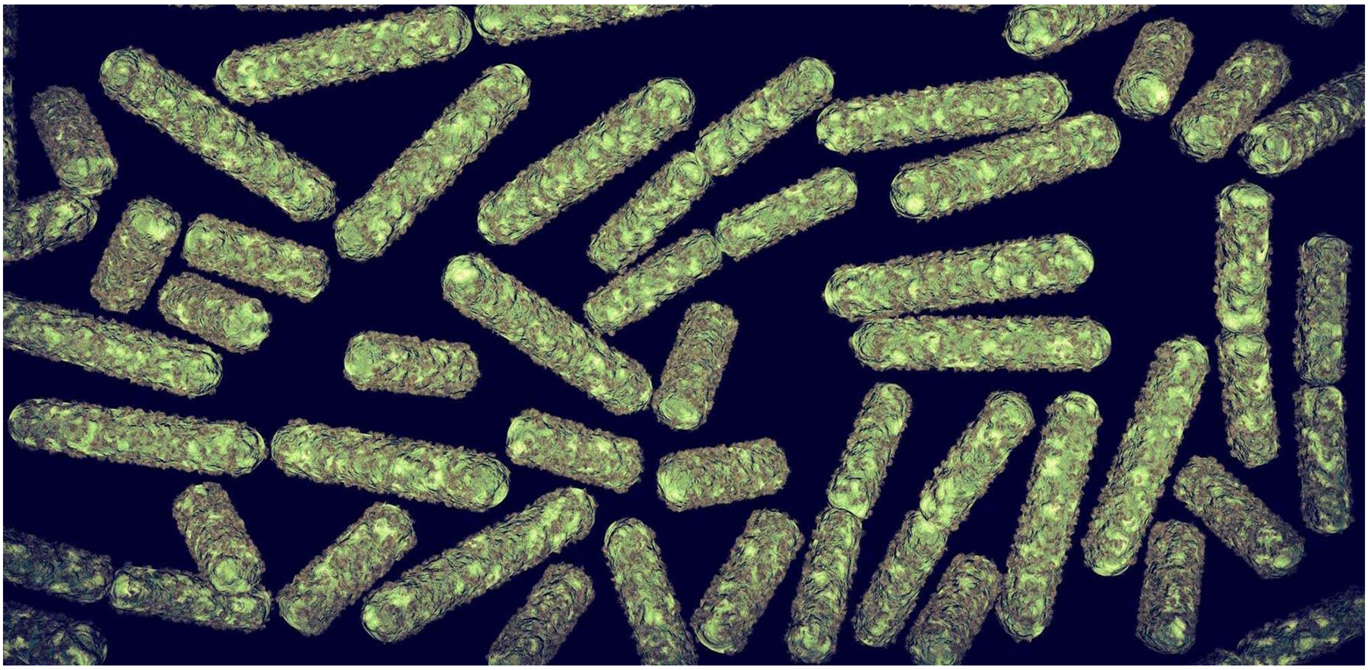
In practice: there is no silver bullet

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



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

Stop endotoxins from decreasing animal performance



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Find out why endotoxemia threatens animal production and how intelligent toxin mitigation solution SOLIS MAX can support endotoxin management.

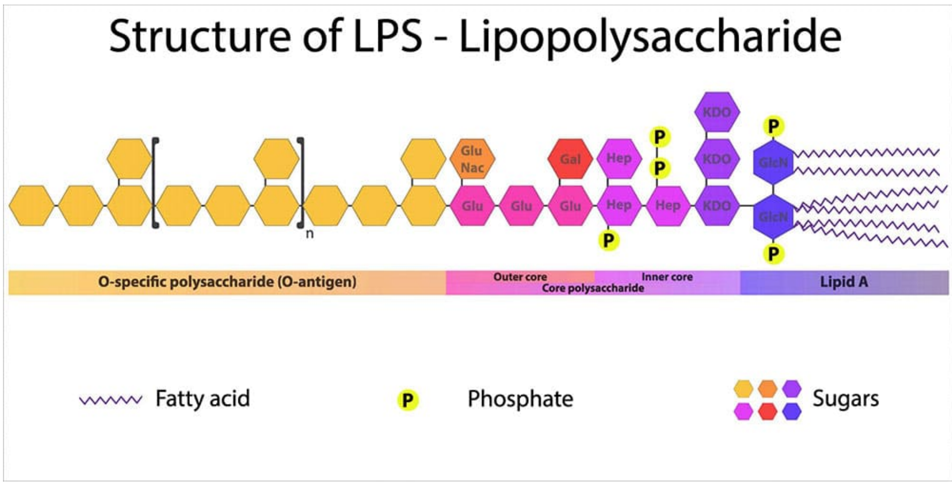

Figure 1: Structure of Lipopolysaccharide

The quick guide to endotoxins (LPS) and what to do about them

Lipopolysaccharides (LPS) are a constant challenge for animal production. LPS, which are also known as endotoxins, are the major building blocks of the outer walls of Gram-negative bacteria (see figure 1). Throughout its life cycle, a bacterium releases these molecules upon cell death and lysis. When endotoxins 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.

LPS may induces inflammation and fever, lowering feed intake, and redirecting nutritional resources to the immune response, which results in hindered animal performance.

Endotoxins depress animal 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 nutrients. An animal also requires a certain baseline amount of nutrients for maintenance, that is, for all activities related to its survival.

As a result of inflammation, endotoxemia leads to a feverish state. Maintenance needs to continue; hence, the energy required for producing heat will be diverted from the nutrients usually spent on production of milk, eggs, meat, etc., and performance suffers. This is amplified because the immune reaction also requires resources (e.g., energy, amino acids, etc. to produce more immune cells).

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.

A piglet study by Huntley, Nyachoti, and Patience (2017) found that LPS-challenged pigs retained 15% less of the available metabolizable energy and showed 25% less nutrient deposition (figure 2). These results illustrate how animal performance declines during endotoxemia.

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

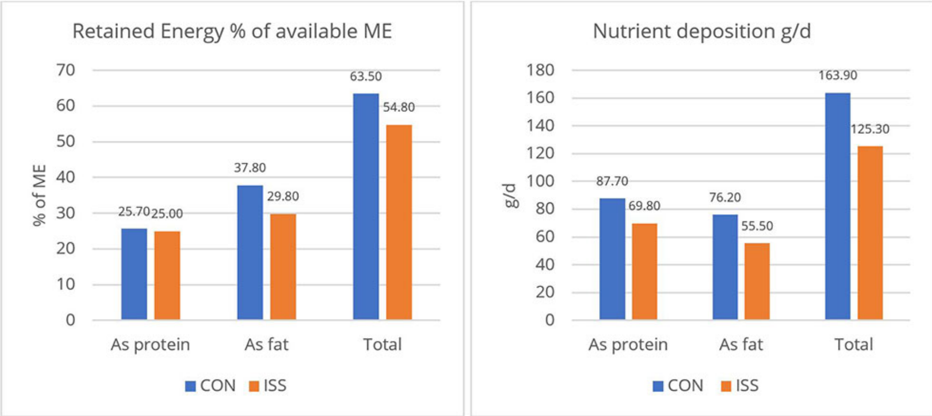

Figure 2: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from Huntley, Nyachoti, and Patience, 2017)

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.

Endotoxin tolerance

The repeated exposure to LPS leads to the production of anti-inflammatory cytokines, as a reaction of the body to prevent tissue damage due to the excessive inflammation. This immunosuppression during stress may lead to an increased risk of secondary infection and poor vaccination titers.

LPS tolerance, also known as CARS (compensatory anti-inflammatory response syndrome) essentially depresses the immune system to control its activity. This “regulation” can be extremely dangerous as an excessive depression of the immune system leaves the organism exposed to the actual pathogens.

The way forward: Natural endotoxin mitigation with SOLIS MAX

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. Stress factors – that are not uncommon in animal production – affect the microbiome (favoring gram-negative bacteria) and also decrease the intestinal barrier function, which leads to the passage of LPS into the bloodstream

Animals suffering from endotoxemia are subject to severe metabolic dysfunctions. If they do not perish from septic shock (and most of them do not), they are still likely to show performance losses. Moreover, they at great risk of immunosuppression caused by CARS, the immune system "overdrive" discussed above.

Fortunately, research shows that EW Nutrition's SOLIS MAX effectively binds bacterial toxins, helping to prevent these scenarios.

In vitro trial shows SOLIS MAX' effectiveness against bacterial endotoxins

Binding endotoxins in the gastrointestinal tract, especially during stress situations in animal production, can help to mitigate the negative impact of LPS on the animals. It reduces the endotoxins passing into the bloodstream and entering the organism.

SOLIS MAX is a synergistic combination of natural plant extracts, yeast cell walls, and natural clay minerals. An *in vitro* study conducted at a research facility in Germany evaluated its binding performance for LPS derived from *E. coli*.

To test the efficacy of SOLIS MAX in binding endotoxins, 0.1% (w/v) of SOLIS MAX was resuspended in endotoxin-free water, with and without a challenge of 25,2568 EU/ml. After one hour, the solutions were centrifuged and the supernatants tested for LPS using Endo-LISA test kits.

The results show that 1 mg of SOLIS MAX adsorbs 20 endotoxin units (EU) of *E. coli* endotoxin, which corresponds – for this challenge – to an 80% adsorption rate (figure 3).

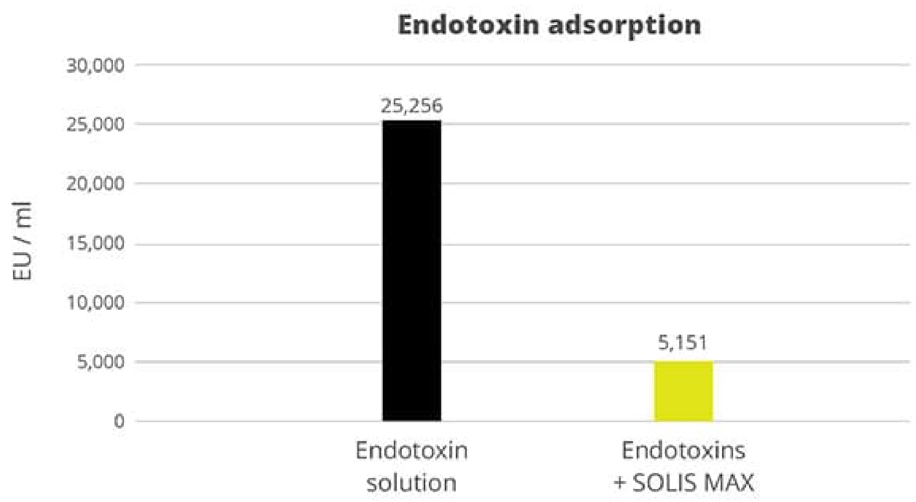

Figure 3: SOLIS MAX effectively adsorbs E. coli endotoxins

Endotoxin solution SOLIS MAX: Stabilize gut health, support performance

The detrimental impact of LPS can be mitigated by using a high-performance solution such as SOLIS MAX. To prevent negative health and performance outcomes for the animal it is important to stabilize the challenged intestinal barrier and to support the balance of the gut microbiome. Binding endotoxins before they can exert their damaging impact is the primary objective, which SOLIS MAX achieves through the intelligent interaction of natural plant extracts. This can be expected to yield positive results in terms of production levels and the prevention of secondary infections, preserving animal health and farms' economic viability.

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