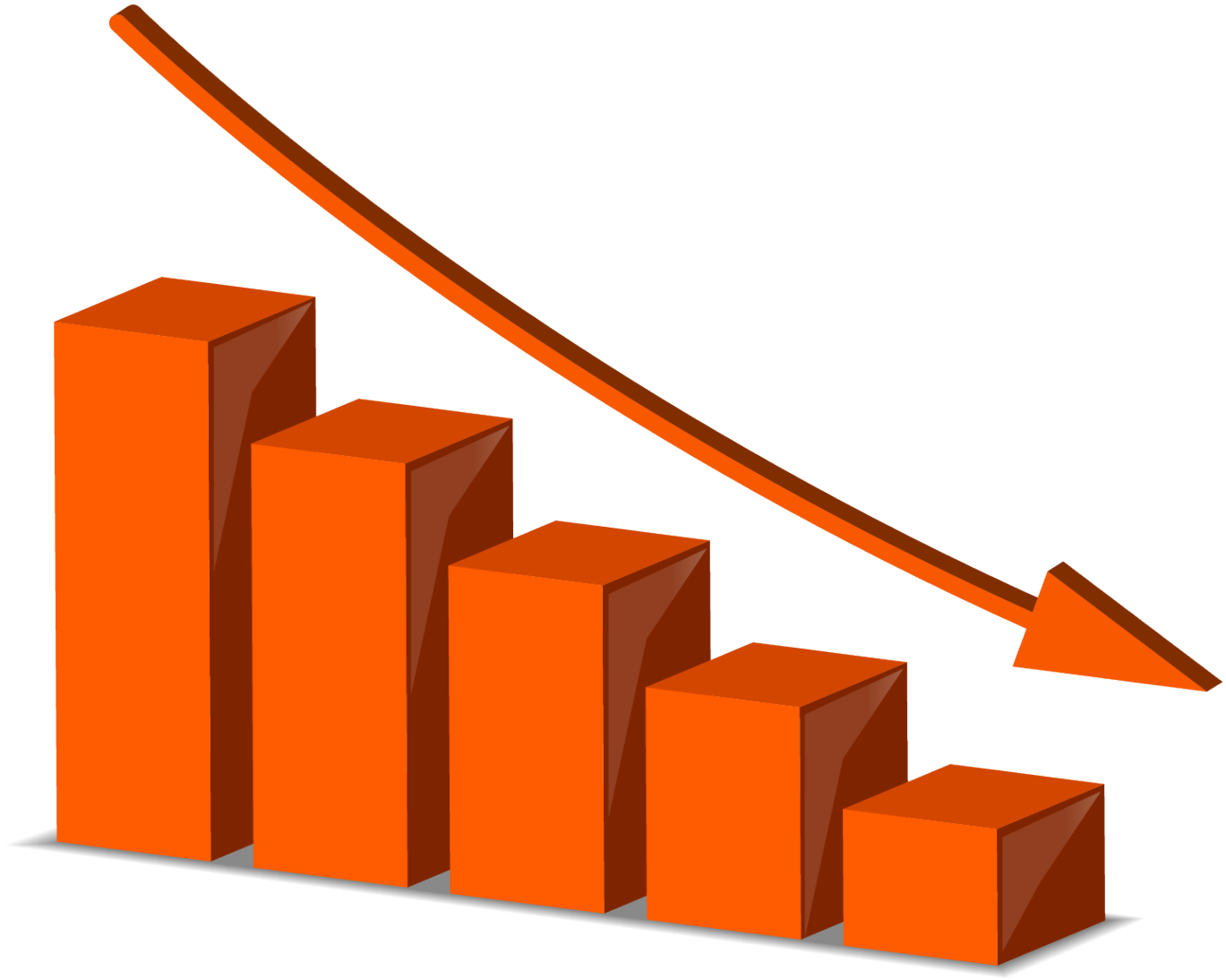
Are endotoxins behind your low livestock productivity?



Find out more about endotoxins here

Impaired health status of the animals in stressful situations or an aggravation of the disease after antibiotic treatment? The culprit might be endotoxins.



What are endotoxins?

Origin

Endotoxins, together with exotoxins, are bacterial toxins. In contrast to exotoxins, which are actively secreted by living bacteria, endotoxins (name “endotoxin” greek; endo = inside; toxin = poison) are components of the outer cell membrane of gram-negative bacteria such as Escherichia coli, Salmonella, Shigella, and cyanobacteria (blue-green algae). They are only released in case of

- bacterial death due to effective host defense mechanism or activities of certain antibiotics
- bacterial growth (shedding) (Todar, 2008-2012)

The location of endotoxins within the bacterial cell © Prof. Dr. med. Marina A. Freudenberg

Structure

Biochemically, endotoxins are lipopolysaccharides (LPS). They are composed of a relatively uniform lipid fraction (Lipid A) and a species-specific polysaccharides chain. Their toxicity is mainly due to the lipid A; the polysaccharide part modifies their activity. Unlike the bacteria, their endotoxins are very heat stable and resist sterilization. The names endotoxin and lipopolysaccharides are used synonymously with “endotoxin” emphasizing on the occurrence and biological activity and “lipopolysaccharide” on the

chemical structure (Hurley, 1995).

General structure of Gram-negative lipopolysaccharides (according to Erridge et al., 2002)

Impact

Endotoxins belong to the so-called pyrogen-agents (they provoke fever), activating several immunocompetent cells' signaling pathways. Early contact with endotoxins leads to activation and maturation of the acquired immune system. Braun-Fahrländer and co-workers (2002) found that children exposed to endotoxins had fewer problems with hay fever, atopic asthma, and atopic sensitization. This might be an explanation that in human populations, after the elevation of the hygiene standards, an increase of allergies could be observed.

Different animal species show different sensibilities to endotoxin infusions, e.g. (healthy) dogs, rats, mice, hens tolerate concentrations ≥1mg / kg body weight, whereas (healthy) ruminants, pigs, horses react very sensible already at concentrations <5µg / kg body weight (Olson et al., 1995 cited in Wilken, 2003).

Reasons for increased exposure of the organism to endotoxins

Endotoxins usually occur in the gut, as the microflora also contains gram-negative bacteria. The precondition for endotoxins to be harmful is their presence in the bloodstream. In the bloodstream, low levels of endotoxins can still be handled by the immune defense, higher levels can get critical. An increase of endotoxins in the organism results from higher input and/or lower clearance or detoxification rate.

Higher input of endotoxins into the organism

The "normal" small amounts of endotoxins arising in the gut due to regular bacterial activity and translocated to the organism have no negative impact as long as the liver performs its clearance function. Also, the endotoxins stored in the adipose tissue are not problematic. However, some factors can lead to a release of the endotoxins or translocation of endotoxins into the organism:

1. Stress

Stress situations such as parturition, surgeries, injuries can lead to ischemia in the intestinal tract and translocation of endotoxins into the organism (Krüger, 1997). Other stress situations in animal production, such as high temperatures and high stocking densities, contribute to higher endotoxin levels in the bloodstream. Stress leads to a higher metabolic demand for water, sodium, and energy-rich substances. For a higher availability of these substances, the intestinal barrier's permeability is increased, possibly leading to a higher translocation of bacteria and their toxins into the bloodstream.

Examples:

- Higher levels of endotoxins in pigs in an experimental study suffering from stress due to loading and transport, elevated temperatures (Seidler (1998) cited in Wilken (2003)).
- Marathon runners (Brock-Utne et al., 1988) and racing horses (Baker et al., 1988) also showed higher endotoxin concentrations in the blood proportional to the running stress; thus, trained horses showed lower concentrations than untrained.

2. Lipolysis for energy mobilization

If endotoxins, due to continuous stress, consistently get into the bloodstream, they can be stored in the adipose tissue. The SR-B1 (Scavenger receptor B1, a membrane receptor belonging to the group of pattern recognition receptors) binds to lipids and the lipopolysaccharides, probably promoting the incorporation of

LPS in chylomicrons. Transferred from the chylomicrons to other lipoproteins, the LPS finally arrives in the adipose tissue (Hersoug et al., 2016). The mobilization of energy by lipolysis e.g., during the beginning of lactation, for example, leads to a re-input of endotoxins into the bloodstream.

3. Damage of the gut barrier

In normal conditions, due to bacterial activity, endotoxins are present in the gut. Damage of the gut barrier allows translocation of these endotoxins (and bacteria) into the bloodstream.

4. Destruction of Gram-negative bacteria

Another "source" for endotoxins is the destruction of the bacteria. This can be done on the one hand by the organism's immune system or by treatment with bactericidal substances targeting gram- bacteria (Kastner, 2002). To prevent an increased release of endotoxins, in the case of Gram-negative bacteria, a treatment with bacteriostatic substances only inhibiting the growth and not destroying the bacteria, or with bactericidal in combination with LPS-binding agents, would be a better alternative (Brandenburg, 2014).

5. Proliferation of gram-negative bacteria

As gram-negative bacteria also release small amounts of endotoxins when they grow, everything promoting their proliferation also leads to an increase of endotoxins:

Imbalanced feeding

High yielder cows e.g., are fed diets rich in starch, fat, and protein. Increased feeding of fat leads to a higher concentration of endotoxins in the organism, as the same "transporter" (scavenger receptor class B type 1, SR-BI) can be used (Hersoug et al., 2016) for the absorption of fat as well as for the absorption of endotoxins.

In a study with humans as representors of the monogastric species, Deopurkar and co-workers gave three different drinks (glucose – 100% carbohydrate, orange juice – 92% carbohydrate, and cream – 100% fat) to healthy participants. Only the cream drink increased the level of lipopolysaccharides in the plasma.

Infectious diseases

Infectious diseases like mastitis, metritis, and other infections caused by gram-bacteria such as E. coli, Salmonella, etc. can be regarded as sources of endotoxin release.

Decreased detoxification or degradation

Main responsible organ: the liver

Task: detoxification and degradation of translocated endotoxin. The liver produces substances such as lipopolysaccharide binding proteins (LBP) which are necessary for binding and neutralizing lipopolysaccharide structures.

During the post-partum period, the organism is in a catabolic phase, and lipolysis is remarkably increased for energy generation due to milk production. Increased lipolysis leads, as mentioned before, to a release of endotoxins out of the adipose tissue but also fatty degeneration of the liver. A fatty degenerated liver cannot bring the same performance in endotoxin clearance than a normal liver (Andersen, 2003; Andersen et al., 1996; Harte et al., 2010; Wilken, 2003). In a study conducted by Andersen and co-workers (1996), they couldn't achieve complete clearance of endotoxins in cows with fatty livers. The occurrence of hepatic lipidoses increases after parturition (Reid and Roberts, 1993; Wilken, 2003).

Also, other diseases of the liver influence endotoxin clearance in the liver. Hanslin and co-workers (2019) found an impaired endotoxin elimination in pigs with pre-existing systemic inflammatory response syndrome.

Relation between lipid metabolism and endotoxin metabolism (according to Fürll, 2000, cited in Wilken, 2003)

Issues caused by endotoxins

Endotoxins, on the one hand, can positively stimulate the immune system when occurring in small amounts (Sampath, 2018). According to McAleer and Vella (2008), lipopolysaccharides are used as natural adjuvants to strengthen immune reaction in case of vaccination by influencing CD4 T cell responses. On the other hand, they are involved in the development of severe issues like MMA-Complex (Pig Progress) or a septic shock (Sampath, 2018).

MMA Complex in sows

MMA in sows is a multi-factorial disease appearing shortly after farrowing (12 hours to three days), which is caused by different factors (pathogens such as E. coli, Klebsiella spps., Staph. spps. and Mycoplasma spps., but also stress, diet). MMA is also known as puerperal syndrome, puerperal septicemia, milk fever, or toxemia. The last name suggests that one of the factors intervening in the disease is bacterial endotoxins. During the perinatal phase, massive catabolism of fat takes place to support lactation. The sows often suffer from obstipation leading to higher permeability of the intestinal wall, with bacteria, respectively endotoxins being transferred into the bloodstream. Another “source” of endotoxins can be the udder, as the prevalence of gram-negative bacteria in the mammary glands is remarkable (Morkoc et al., 1983).

The endotoxins can lead to an endocrine dysfunction: ↑ Cortisol, ↓ PGF2α, ↓Prolactin, ↓ Oxytocin. MMA stands for:

– Mastitis, a bacterial infection of the udder.

Mastitis can be provoked from two sides: on the one hand, endotoxemia leads to an elevation of cytokines (IL1, 6, TNFα). Lower Ca- and K-levels cause teat sphincter to be less functional, facilitating the entry of environmental pathogens into the udder and resulting in mastitis. On the other hand, due to farrowing stress, Cortisol concentrations get higher. The resulting immunosuppression enables E. coli to proliferate in the udder.

– Metritis, an infection of the uterus with vulvar discharges:

It leads to reduced contractions and, therefore, to prolonged and/or complicated farrowing or dead piglets. Metritis can be promoted by stress causing a decrease in oxytocin and prostaglandin F2α secretion. Morkoc and co-workers (1983) didn’t find a relation between metritis and endotoxins.

– Agalactia, a reduction or total loss of milk production:

In many cases, agalactia is not detected until the nursing litter shows signs of hunger and/or weight loss. At worst, the mortality rate in piglets increases. Probably, milk deficiency is caused by lower levels of the hormones involved in lactation. Prolactin levels e.g., may be dramatically reduced by small volumes of endotoxin (Smith and Wagner, 1984). The levels of oxytocin are often half those in normal sows (Pig Progress, 2020).

Endotoxin shock

A septic shock can be the response to the release of a high amount of endotoxins.

In the case of an infection with gram-negative bacteria, the animals are treated with (often bactericidal) antibiotics. Also, the immune system is eliminating the bacteria. Due to bacterial death, endotoxins are massively released. When not bound, they activate the immune system including macrophages, monocytes, and endothelial cells. Consequently, high amounts of cellular mediators like TNFα, Interleukin

1 (IL-1), IL-6, and leukotrienes are released. High levels of pro-inflammatory cytokines activate the complement and coagulation cascade. In some animals, then the production of prostaglandins and leukotrienes is stimulated, implicating high fever, decreased blood pressure, generation of thrombi in the blood, collapse, damaging several organs, and lethal (endotoxic) shock.

Endotoxic shock only occurs to a few susceptible animals, although the whole herd may have been immune-stimulated. A more severe problem is the decrease in the normally strong piglets' performance, deviating resources from production to the immune system because of the endotoxemia.

Amplified diarrhea

Lipopolysaccharides lead to an augmented release of prostaglandins, which influence gastrointestinal functions. Together with leukotrienes and pro-inflammatory mediators within the mucosa, they reduce intestinal absorption (Munck et al., 1988; Chiossone et al., 1990) but also initiate a pro-secretory state in the intestine. Liang and co-workers (2005) observed a dose-dependent accumulation of abundant fluid in the small intestine resulting in increased diarrheagenic activity and a decreased gastrointestinal motility in rats.

Conclusion

Acting against Gram- bacteria can result in an even more severe issue – endotoxemia. Endotoxins, besides having a direct negative impact on the organism, also contribute to some diseases. Supporting gut health by different approaches, including the binding of toxins, helps to keep animals healthy.

By Inge Heinzl, EW Nutrition

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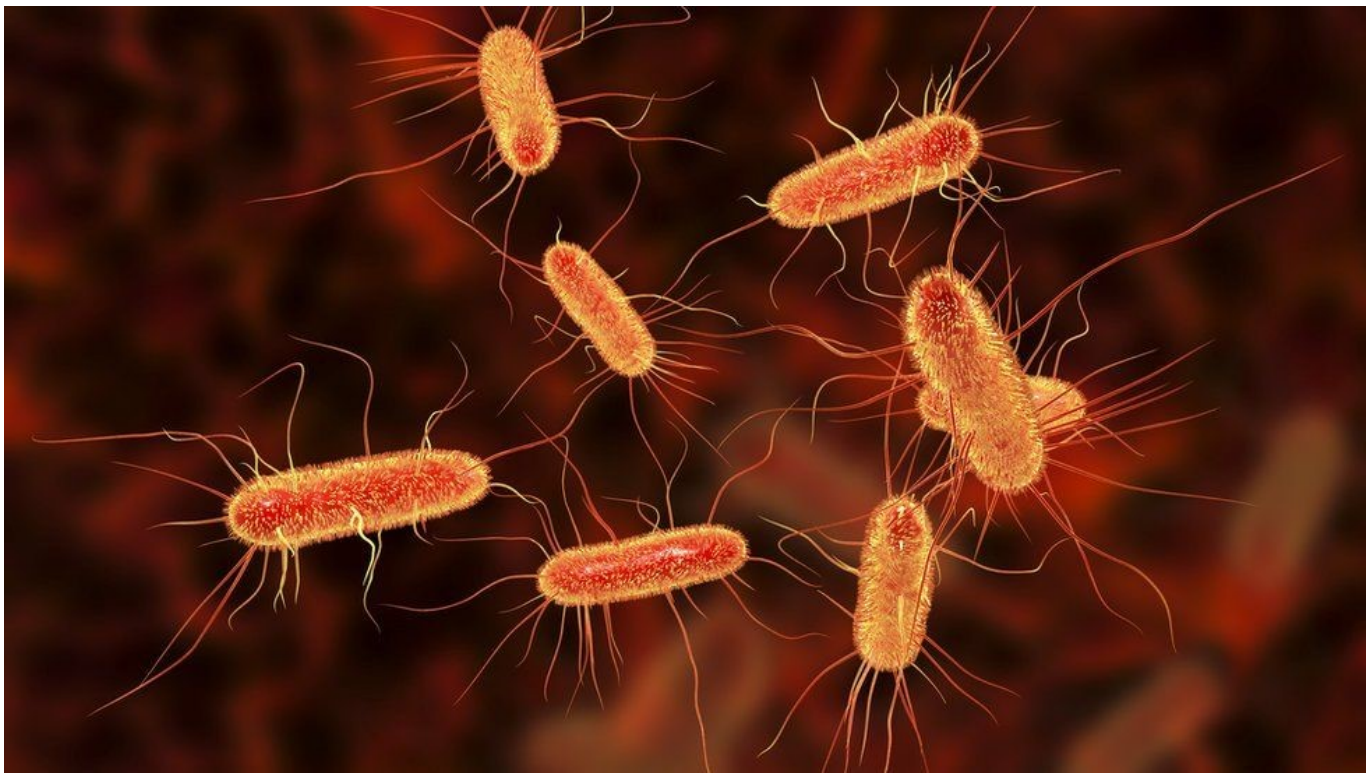
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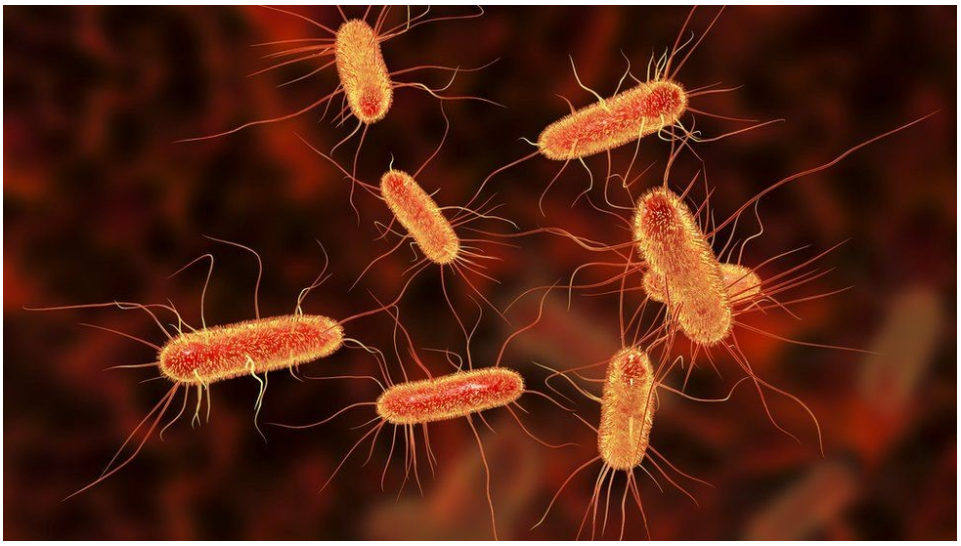
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The hidden danger of endotoxins in animal production



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).

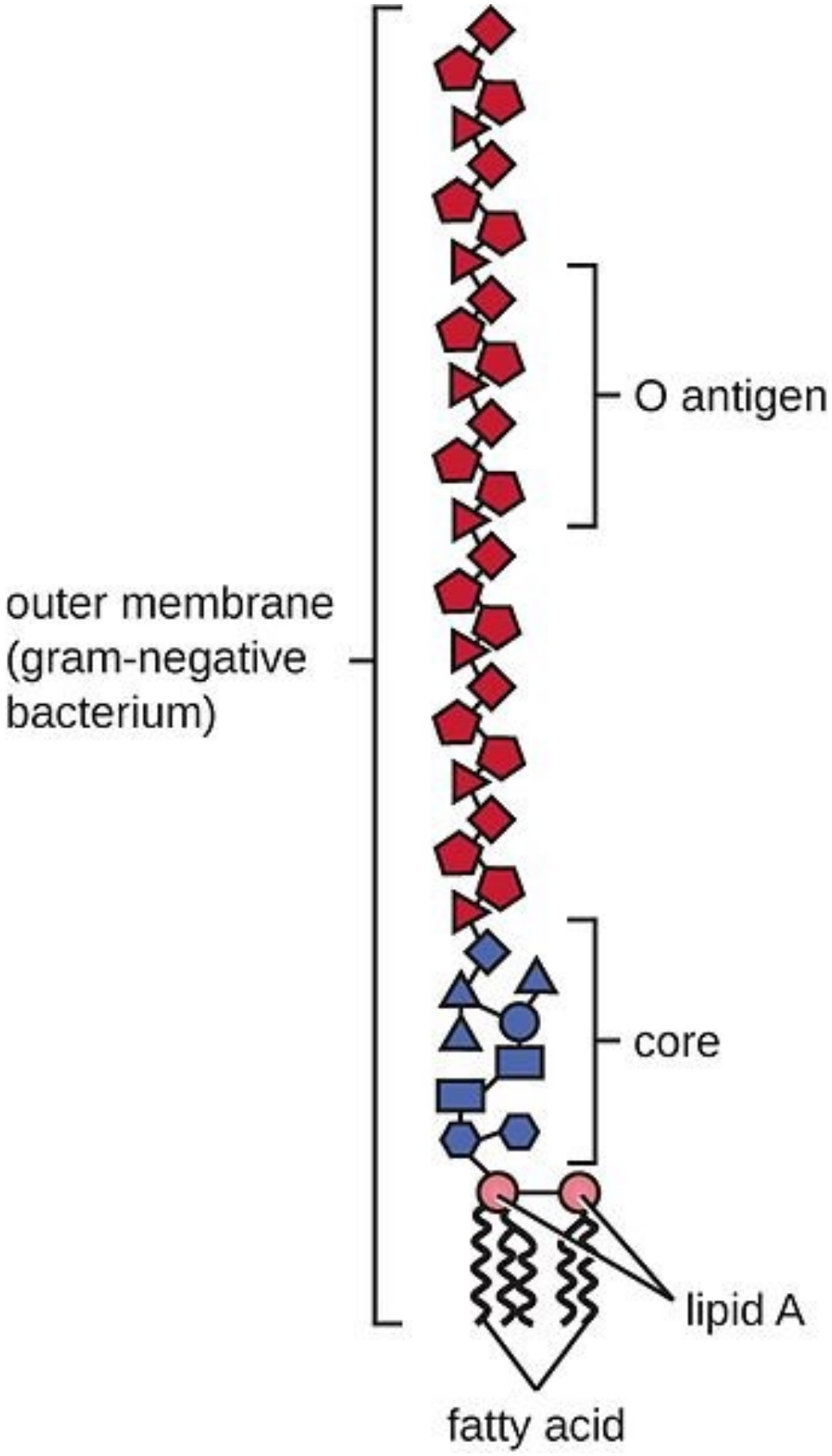


Figure 1: Structure of an LPS

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 ≈830 µm in

the colon and ≈123 µm in the jejunum), vulnerable points exist (cf. Zachary 2017).

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

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

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

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

LPS' modes of action

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

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

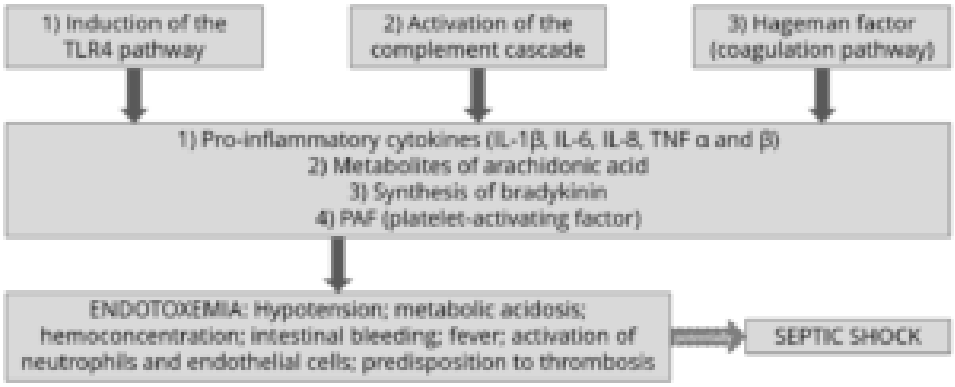


Figure 2: How LPS leads to endotoxemia – 3 modes of action

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

- **Pro-inflammatory cytokines** (see above) modulate the functional expression of other immune cell types during the inflammatory response;
- **Metabolites of arachidonic acid** (prostaglandins, leukotrienes, and lipoxins), intra- and extracellular messengers that influence the coagulation cascade;

- Synthesis in the blood of **bradykinin**, a peptide responsible for the typical symptoms of inflammation, such as swelling, redness, heat and pain;
- **PAF** (platelet-activating factor), which creates inflammatory effects through narrowing of the blood vessels and constriction of the airways, but also through the degranulation of leukocytes.

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

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

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

2) Abnormalities in body temperature

3) Progressive hypoperfusion at the level of the microvascular system

4) Hypoxic damage to susceptible cells

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

5) Quantitative changes in blood levels of leukocytes and platelets

6) Disseminated intravascular coagulation (see Hageman factor)

7) Multi-organ failure

8) Death of animal

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

Farm animal research on endotoxemia pathogenesis

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

How the metabolic effects of endotoxemia depress performance

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

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

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

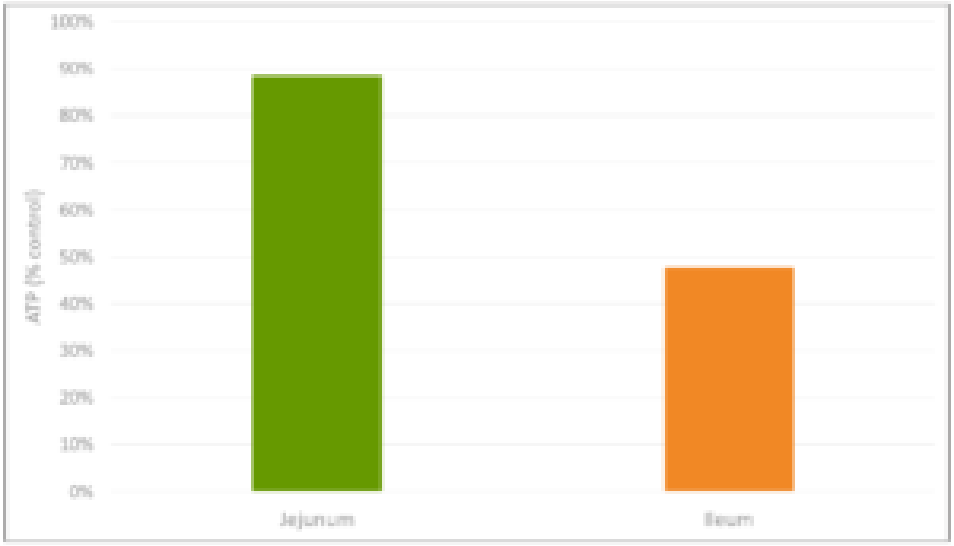


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from Li et al., 2015)

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

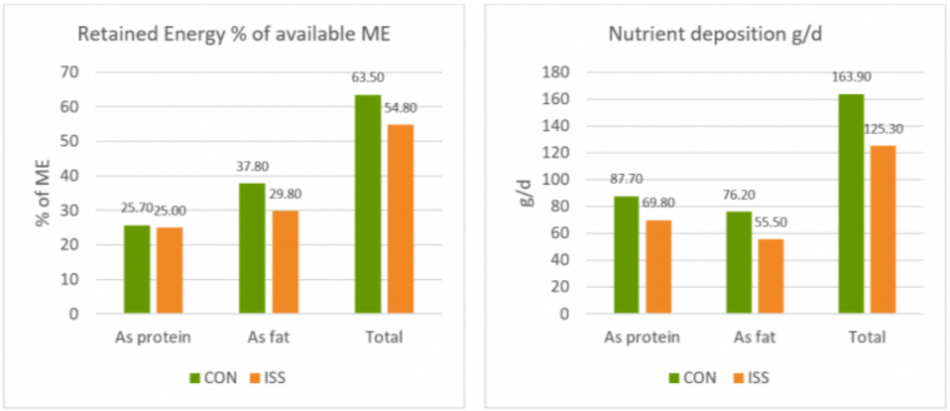


Figure 4: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from Huntley, Nyachoti, and Patience, 2017)

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

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

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

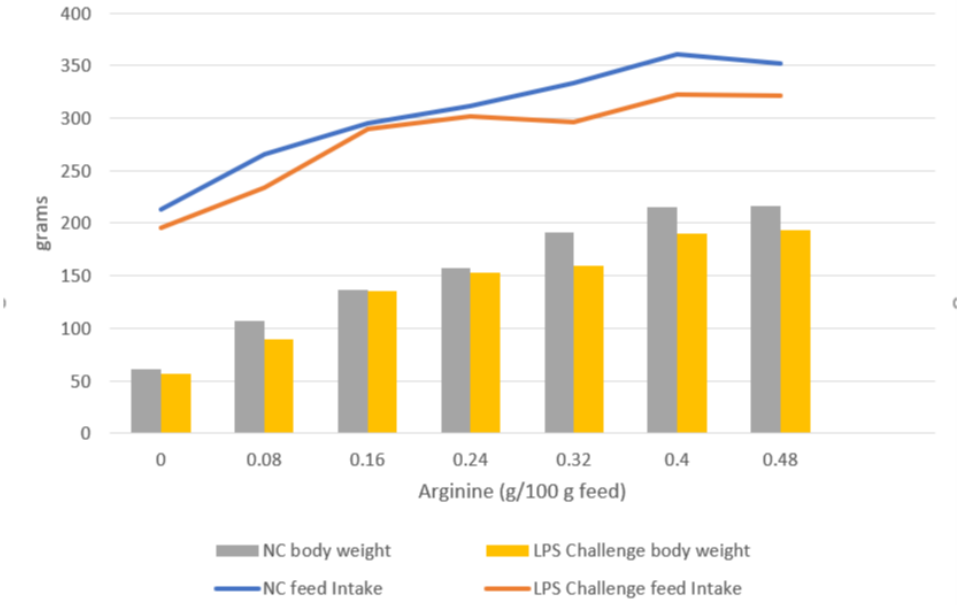


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on Webel, Johnson, and Baker, 1998)

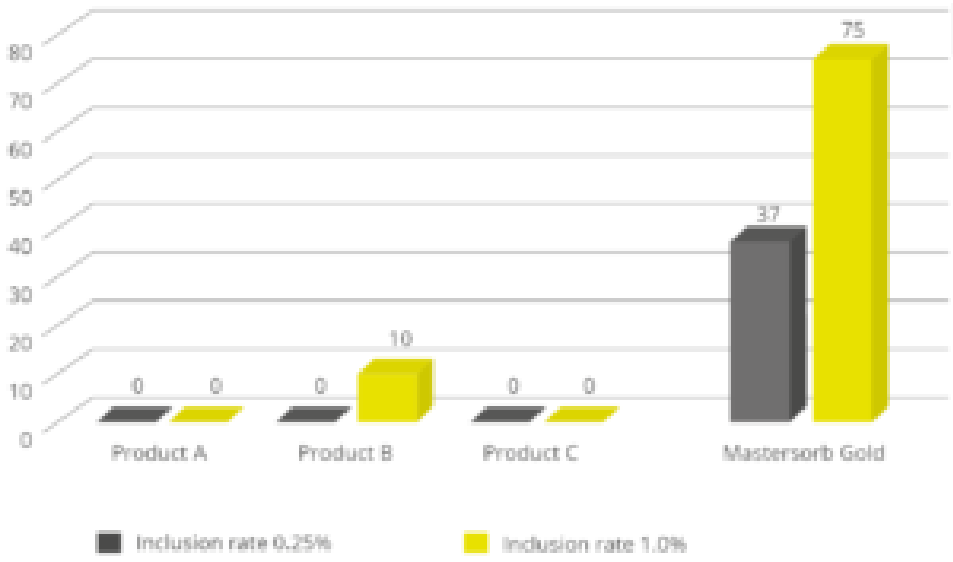
NC = negative control

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

The way forward: Endotoxin mitigation

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

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



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

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

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