From sub-acute ruminal acidosis to endotoxins: Prevention for lactating cows

by Technical Team, EW Nutrition

Sub-acute acidosis (SARA) is linked to high levels of ruminal LPS. The LPS cause inflammation and contribute to different metabolic conditions and diseases. Various strategies and solutions can be applied to modulate the rumen microbiota and prevent this risk.

In sub-acute rumen acidosis (SARA), the quantity of free lipopolysaccharides (LPS) coming from Gram- bacteria increases considerably. These LPS cross the ruminal wall and intestine, passing into the bloodstream. The negative consequences on the health of the animal are then reflected in decreased productive and reproductive performance.

The LPS are released during the lysis of GRAM- bacteria which die due to the low pH, and these bacteria are mainly responsible for the production of propionic acid for the energy yield that is obtained. It is essential to preserve ruminal balance between Gram+ and Gram- such that there is no excess of LPS.

Nutritional needs of lactating cows with SARA

In the first phase of lactation (from 1 week after calving to 80 – 100 days of lactation), the cow needs a high energy level to meet the large demand for milk production. This energy demand is often not fully satisfied and feed intake falls short. This deficit leads to the need to provide as much energy as possible per feed ration.

Imagine a 650 kg live weight cow, producing about 35 kg of milk per day with a fat percentage of 3.7 and a protein percentage of 3.2. To achieve this production level and fulfill its maintenance requirements, this animal needs a feed intake of 22 kg of dry matter (DM) per day, with an energy level of 21 UFL equal to 36,000 Kcal/day of NE l (Net Energy Lactation)).

To obtain an energy supply of this type, it is necessary to provide rations with a high content of cereals rich in nonstructured carbohydrates (NSC). This will allow the animals to obtain the maximum efficacy in getting the NE l from the metabolizable energy (ME) expressed as kl*.

*kl expresses the effectiveness in passing from EM to EN l net of the heat dissipated by the animal, therefore kl = ENl/EM (Van Es 1978).

Compared to a diet rich in NDF (Neutral Detergent Fiber), this type of diet promotes and stimulates certain strains of bacteria to the detriment of others, shifting the balance towards a greater population of bacteria that produce propionic acid instead those which produce acetic acid. This change also determines a greater share of Gram- compared to Gram+.

What is rumen acidosis?

Rumen acidosis is that “pathology” whereby the volume of SCFA (Short Chain Fatty Acids) produced by the rumen bacteria is greater than the ability of the rumen itself to absorb and neutralize them. Rumen acidosis is mainly caused by the amylolytic and saccharolytic bacteria (*Streptococcus bovis; Selenomonas ruminantium, Bacteroides amylophilus, Bacteroides ruminicola* and others) responsible for the production of lactic acid. Unlike the other most representative volatile fatty acids (acetic, butyric and propionic), lactic acid has a lower pKa: 7 (3.9 versus 4.7). This means that for the same amount of molecules produced, lactic acid releases a number of ions H^+ in the fluid ten times greater than other VFAs, with evident effects on the pH.

Ruminal acidosis can be characterized as acute or subacute. During acute ruminal acidosis, the pH in the rumen drops below 4.8 and remains low for an extended period of time. Acute acidosis leads to complete anorexia, abdominal pain, diarrhea, lethargy, and eventually death. However, the prevalence of acute acidosis in dairy is very low.

Consequences of rumen acidosis

In such situations, a series of negative consequences can be triggered in the lactating cow. Investigations (for instance, using fistulated cows) can reveal, among others, the following alteration in the rumen:

- Shift in total microbiome rumen profile (density; diversity; community structure)
- Shift in protozoa population (increase in ciliates protozoa after 3 weeks of SARA; increase in the GNB population)
- Shift in fungi population (decreasing the fungi population with high fibrolytic enzymes, which are sensitive to low pH)
- Rise in LPS rumen concentration (increasing the GNB strain and their lysis)
- Influence on the third layer of Stratified Squamous Epithelium (SSE) (desmosomes and tight junctions)
- Lower ruminal fiber degradation (reduction in the number of cellulolytic bacteria which are less resistant to acid pH)
- Reduction of the total production of fatty acids (propionic, acetic, butyric), therefore less available energy
- Lower rumen motility (also as a consequence of the smaller number of protozoa)
- The increased acid load damages the ruminal epithelium
- Acid accumulation increases the osmotic pressure of the rumen inducing an higher flux of water from the blood circulation into the rumen, causing swelling and rupture of rumen papilla as well as a greater hemoconcentration

The last points are extremely important, as it enables an easier passage of fluids from the blood to the pre-stomachs, greatly influencing the fermentation processes.

Furthermore, with diets low in NDF, the level of chewing and salivation is certainly lower, with a consequent lower level of salivary buffers that enter the rumen and which would maintain an appropriate pH under normal conditions.

Rumen sub-acute and acute acidosis: a fertile ground for LPS

Studies inducing SARA in dairy cows have shown that feeding high levels of grain causes the death and cell lysis of Gram- bacteria, resulting in higher concentration of free LPS in the rumen. In a trial conducted by Ametaj et al., in 2010 (Figure 1), a lower ruminal pH and an increase in the concentration of LPS in the rumen fluid -measured as ng / ml (nanograms / milliliter)-, was the result of increasing of NSC present in the diet (% of grains).

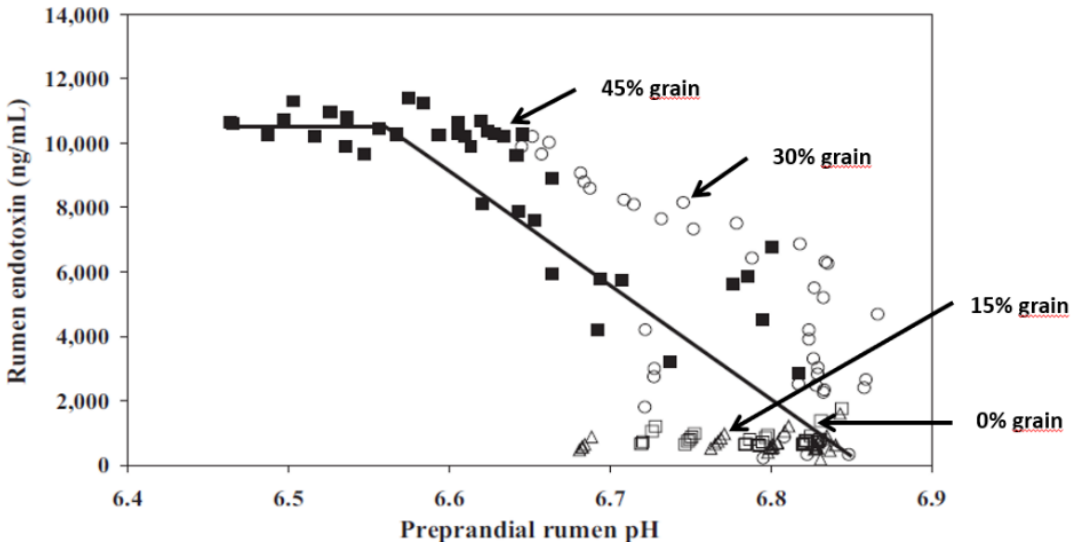


Figure 1. The increase in the level of endotoxins in the rumen is directly correlated with an increase in ration concentrates

In the rumen, the presence of Gram- is very significant, however the dietary changes towards high energy concentrates, reduce the substates necessary for them to thrive, leading to their lysis and favoring gram-positive bacteria (Gram+). Gram+ also produce bacteriocins against a wide variety of bacteria, including many Gram-. Figure 2 shows the influence of ruminal pH in the population of different bacteria, many of which are are crucial for the production of SCFA and therefore of energy.

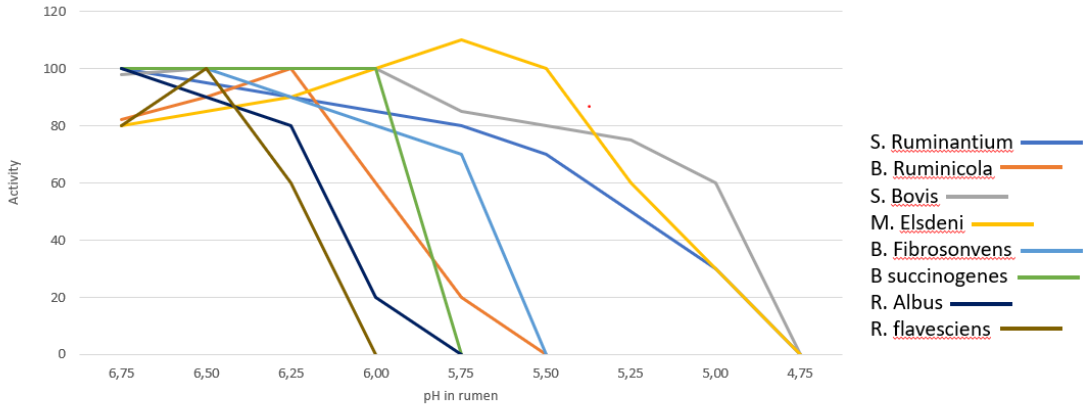


Figure 2. Activity of main bacteria in the rumen in function of pH (Daniele Cevolani Edizioni Agricole di New Business Media srl 2020)

It is therefore necessary to pay close attention to the energy level of the ration as an energy input (generally around 1500 – 1700 Kcal/kg of DM intake). At the same time, we need to ensure that the animal does receive and ingest that daily amount of DM. If ingestion is negatively influenced by acidosis (clinical or sub-clinical), this can lead to endotoxemia, with harmful consequences for the animal's health and production performance.

We can therefore note that the level of LPS (endotoxins) present in the rumen is directly correlated with the pH of the rumen itself and with a symptomatologic picture dating back to SARA. This occurs when the mortality and lysis of Gram- bacteria (GNB) is high and through the consequent imbalance created with diets containing excess fermentable starches, compared to diets with higher fiber content.

In fact, it was shown that the transition from a concentrated fodder ratio of 60:40 to a more stringent ratio of 40:60 caused the level of free LPS in the rumen to go from 410 to 4.310 EU / ml.

Endotoxemia: Pathological consequences in dairy cows

Once the LPS enter the bloodstream, they are transported to the liver (or other organs) for the detoxification. However, sometimes this is not enough to neutralize all the endotoxins present in blood. The remaining excess can cause issues such as the modification of the body's homeostasis or cause that cascade of inflammatory cytokines responsible for the most common pathologies typical in cows in the first phase of lactation. The most common symptoms are the increase of somatic cells in milk or claws inflammation.

Pro-inflammatory cytokines as TNF, IL6 and IL8 induced by LPS-related inflammation are able to stimulate the production of ACTH (adrenocorticotropic hormone).

ACTH, together with cortisol and the interleukins, inhibit the production of GnRH and LH, with serious effects on milk production. The productivity and the fertility of the animal are thus compromised.

Moreover, prostaglandins are as well stimulated by LPS, and are linked with fever, anorexia and ruminal stasis. This not only limits the amount of energy available for production and maintenance functions, but also induces a higher susceptibility to disease and adds-up to the emergence of other metabolic conditions, such as laminitis and mastitis.

Preventing rumen acidosis

The solution to these massive risks is a prudent and proactive approach by the nutritionist towards all situations that can cause a rapid increase of Gram- in the rumen. It is therefore necessary to avoid cases of clinical and sub-clinical acidosis (SARA) in order to avoid the issues listed above. This would also help avoid stressful conditions for the animal that would lead to decreased performance and health.

To maintain balance and a healthy status of the animal, the use of additives such as phytomolecules and binders is suggested in the first phase of lactation, starting from 15 days before giving birth.

Activo Premium (a mix of phytogenic substances) has given excellent results in decreasing the acetic/propionic acid ratio, while safeguarding the population of Gram+ bacteria. This is in contrast to treatments with ionophores, which, as is well known, interfere with the Gram+ population.

Case study. Acetic acid:propionic acid ratio with Activo Premium

In a study conducted at the the University of Lavras and the Agr. Res. Comp. of Minas Gerais (both Brazil), 30 Holstein cows were allocated to two groups considering parity and milk production. One group was fed the standard feed (control), the other group received standard feed containing 150mg of Activo Premium/kg of dietary dry mass (DM). The following parameters were measured or calculated: intake of DM and milk production, milk ingredients such as fat, protein, lactose every week, body weight and body condition score every two weeks, and ruminal constituents (ph and SCFAs) through oesophaeal samples at day 56.

Activo Premium was able to decrease the ratio between acetic acid and propionic acid, and at the same time maintain the level of Gram+ bacteria in the rumen, thus reducing the risk of endotoxins. The same trial carried out at the University of Lavras demonstrated how the performance of the animals was superior in the group fed with Activo Premium compared to the control group (see below).

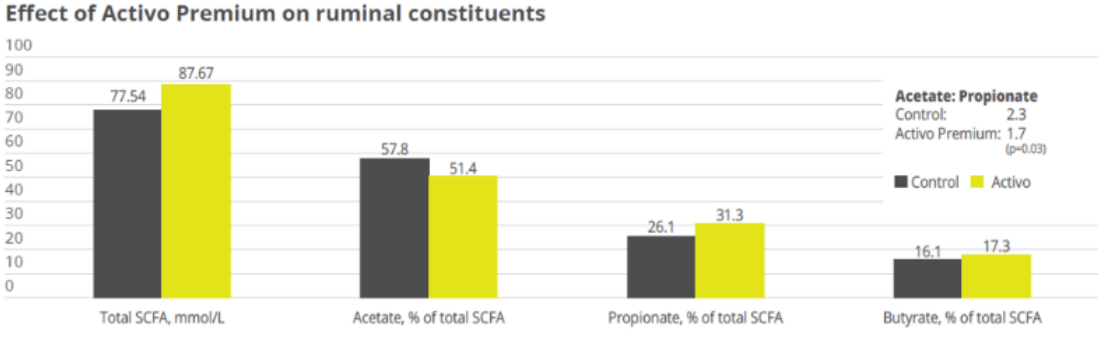


Figure 3. Effect of Activo Premium on ruminal constituents

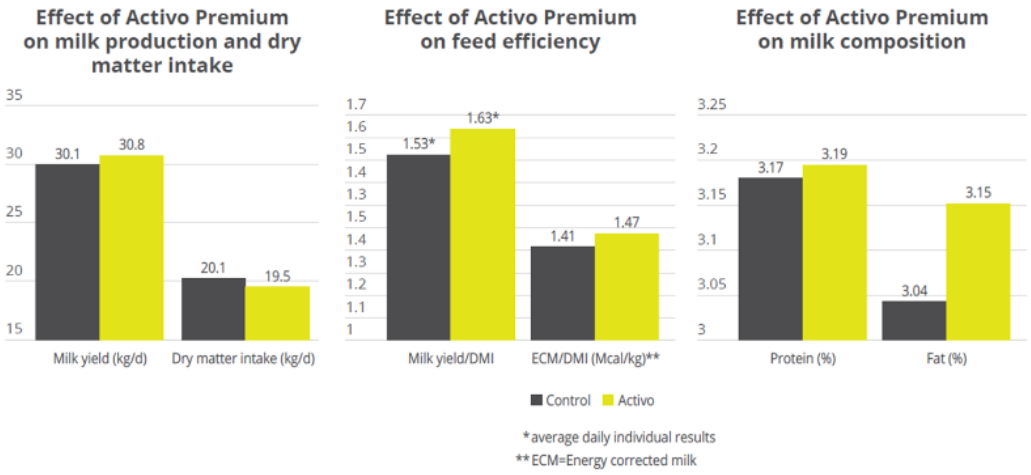


Figure 4. Effect of Activo Premium on animal performance

Solution: Preserve Gram+ bacteria levels while decreasing free LPS

We have therefore seen how important it is to decrease the acetic:propionic ratio in the rumen to obtain a greater share of available energy. However, the level of endotoxins in the rumen must remain low in order to avoid those problems of endotoxemia linked to very specific pathologies typical of "*super productive cows*". These pathologies (always linked to inflammatory manifestations) can be prevented by decreasing the level of free LPS in the rumen with a product that can irreversibly bind the LPS and thus make them inactive.

In a trial with porcine intestinal cells (IPEC-J2) challenged by E. coli LPS, a decrease in the intensity of inflammation was observed when Mastersorb Gold was added. This decrease could be shown through a lower amount of phosphorylated NF-kB in an immunofluorescence trial, as well as through the reduced production of Interleukin (IL)-8 in the cells measured by ELISA.

The fact that pig intestine tissue was used does not affect the adsorption concept. In this case, these intestinal cells are only a vehicle to demonstrate that in an aqueous solution containing 50 ŋg of LPS / ml and in the same solution with the addition of Mastersorb Gold, the level of LPS actually active is decreased, as a part of the LPS was tied up by Mastersorb. The solution with a lower level of LPS gave minor "inflammatory" reactions to intestinal cells, and this can be statistically reported in dairy cows.

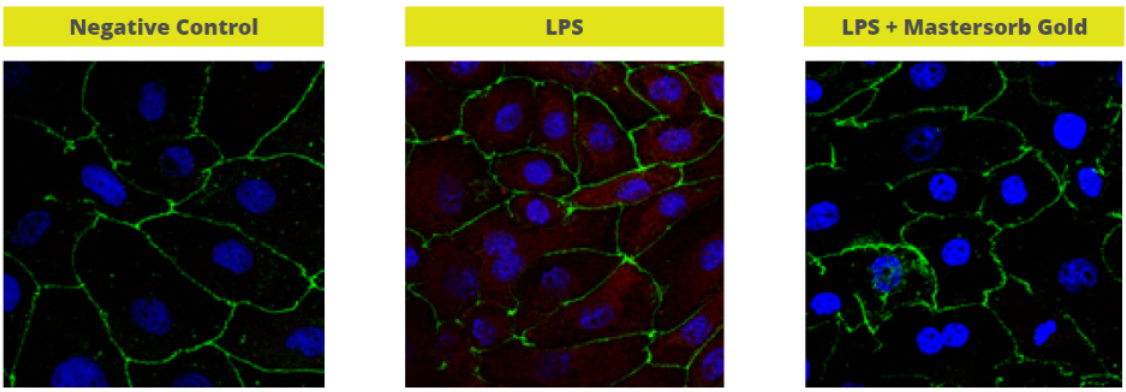


Figure 5. Immunofluorescence in PEG-J2: Challenge with LPS without (in the middle) and with Mastersorb Gold (right)

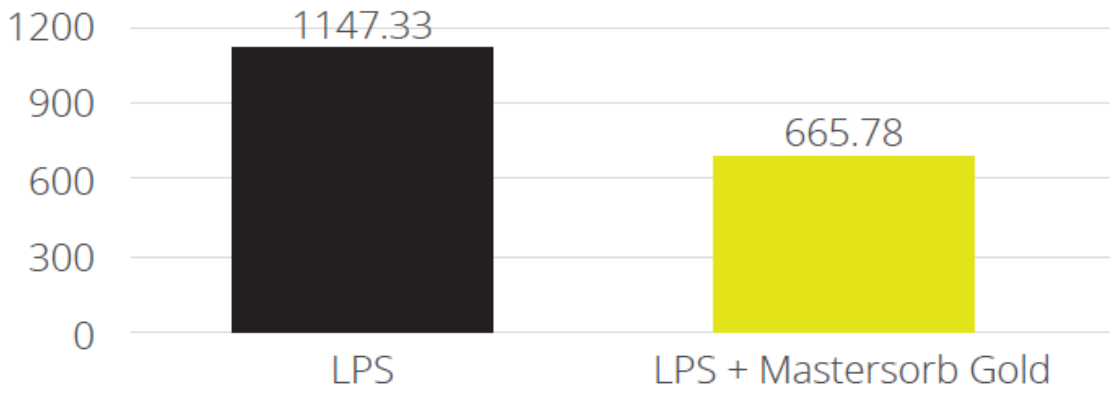


Figure 6. IL-8 AP secretion after incubation with LPS 0111:B4 for 24h without and with Mastersorb Gold

Conclusions

To demonstrate how the decrease in the level of LPS in the rumen is directly correlated with inflammatory states in general, a trial with a total of 60 dairy cows shows that the inclusion of 25g of Mastersorb Premium/animal/day increases milk yield and improves milk quality by decreasing somatic cell count. Adsorbing substances contained in Mastersorb Premium tie up the LPS produced in the rumen in different cow lactation phases.

Normally, the rise in the level of somatic cells in milk depends on etiological agents such as *Streptococcus spp, Staphylococcus spp, mycoplasma* and more. LPS stress is not the sole agent responsible for raising somatic cell counts, but also other factors among which:

- Lactation stage and age of the animal
- Season of the year (in summer the problem is increased)
- Milking plant (proper maintenance)
- General management and nutrition

However, by reducing the level of LPS, Mastersorb provides an important aid to decrease somatic cell count.

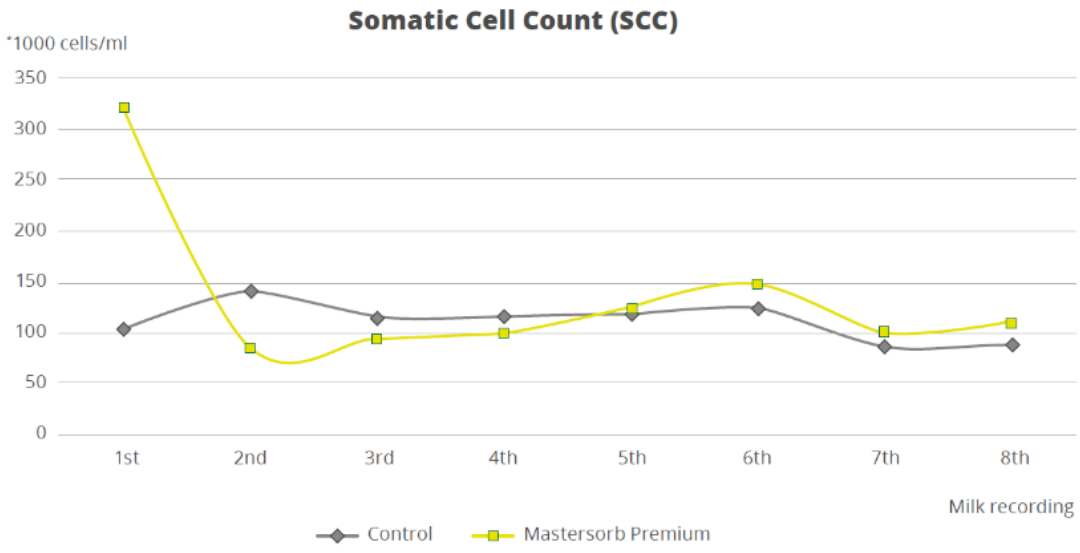
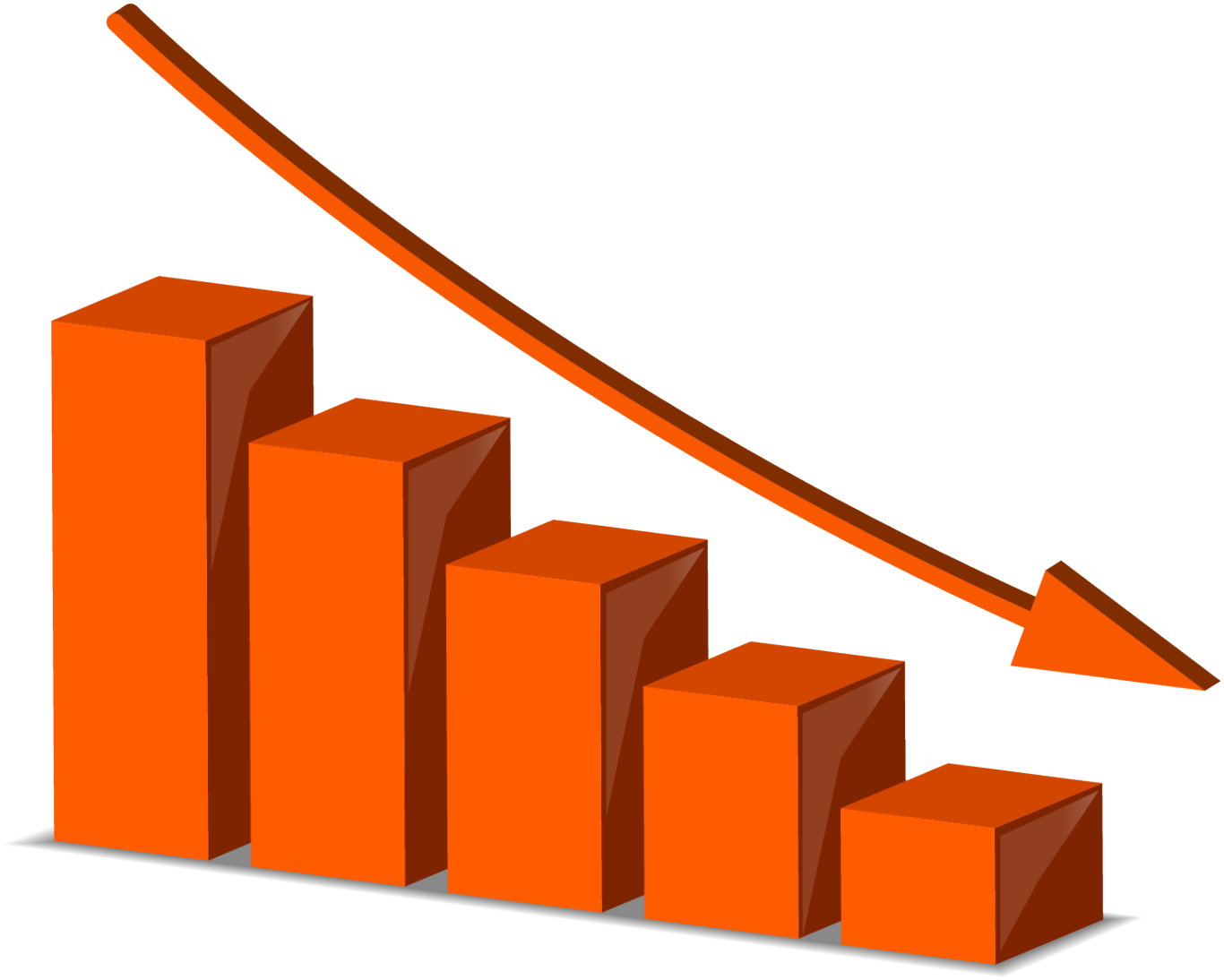


Figure 7. Effect of Mastersorb Premium on somatic cell count

Prevent escalation with rumen balance

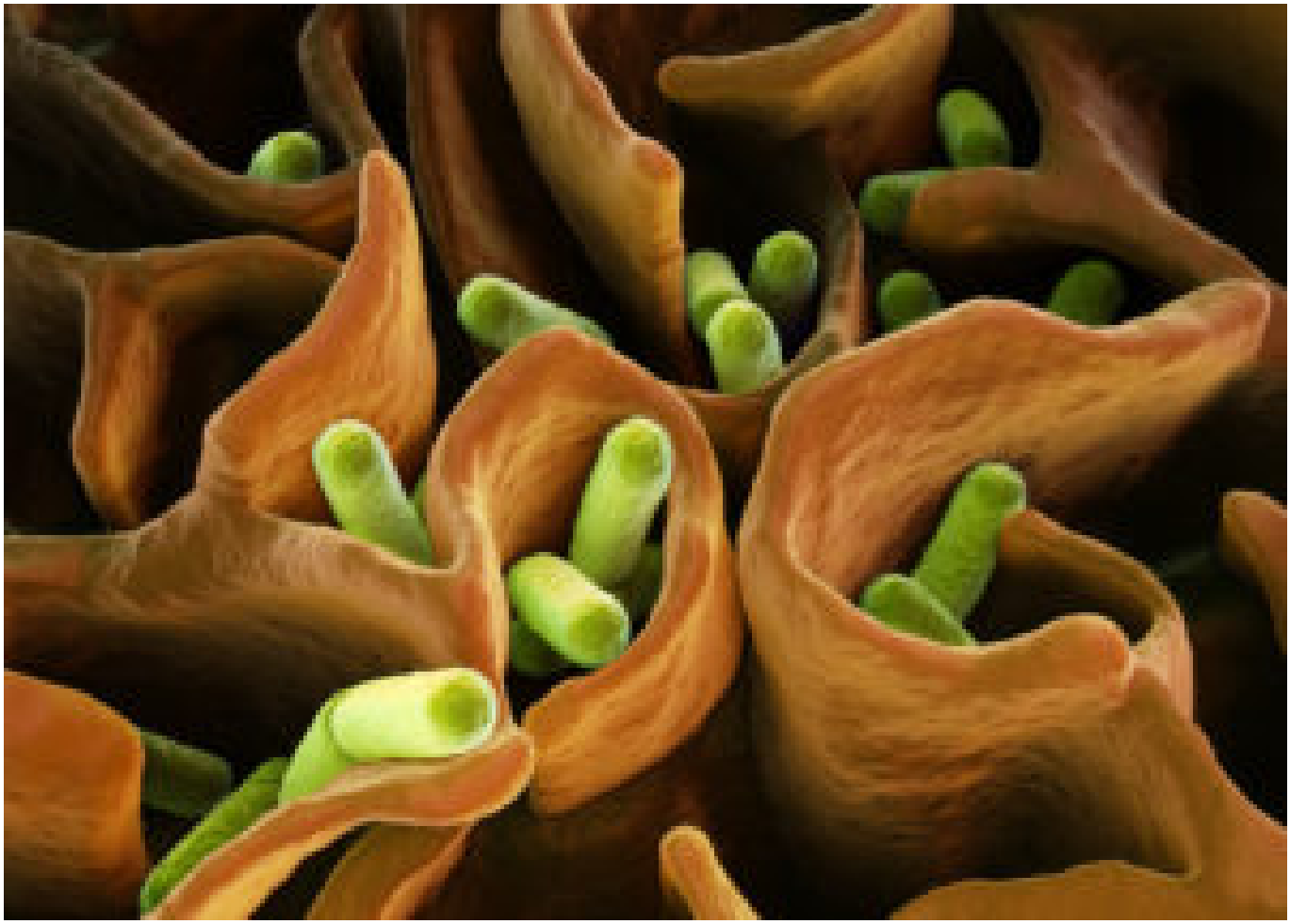
In the end, ruminant producers are, like all livestock operations, interested in producing healthy animals that can easily cope with various stressors. Ensuring a proper diet, adjusted to the energy requirements of various production stages, is a first step. Providing the animal with the ingredients that modulate the microbiota and reduce the negative impact of stress in the rumen is the next essential step in efficient production.

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

References

Andersen, P.H. "Bovine endotoxicosis – some aspects of relevance to production diseases. A review." *Acta vet. scand*. Suppl. 98 (2003): 141-155. DOI: 10.1186/1751-0147-44-S1-P57

Andersen, P.H., N. Jarløv, M. Hesselholt, and L. Bæk. "Studies on in vivo Endotoxin Plasma Disappearance Times in Cattle." *Zentralblatt für Veterinärmedizin*. Reihe A 43 no. 2(1996): 93-101. DOI: 10.1111/j.1439-0442.1996.tb00432.x

Baker, B., S.L. Gaffin, M. Wells, B.C. Wessels and J.G. Brock-Utne. "Endotoxaemia in racehorses following exertion." *Journal of the South African Veterinary Association* June (1988): 63-66. https://journals.co.za/docserver/fulltext/savet/59/2/1341.pdf?expires=1598542211&id=id&accname=guest&checksum=E50C766D318776E09CA41DA912F14CAD

Beutler, B. and T. Rietschel. "Innate immune sensing and its roots: The story of endotoxin." *Nature Reviews / Immunology* 3(2003): 169-176. DOI: 10.1038/nri1004

Brandenburg, K. "Kleines Molekül – große Hoffnung – Neue Behandlungsmöglichkeit gegen Blutvergiftung in Sicht." Newsletter 70 (Okt.); Bundesministerium für Bildung und Forschung (2014). https://www.gesundheitsforschung-bmbf.de/de/kleines-molekul-grosse-hoffnung-neue-behandlungsmoglichkeit-gegen-blutvergiftung-in-sicht-2716.php

Braun-Fahrländer, C., J. Riedler, U. Herz, W. Eder, M. Waser, L. Grize, S. Maisch, D. Carr, F. Gerlach, A. Bufe, R.P. Lauener, R. Schierl, H. Renz, D. Nowak and E. von Mutius. „Environmental exposure to endotoxin and its relation to asthma in school-age children. "*The New England Journal of Medicine* 347 (2002): 869-877. DOI: 10.1056/NEJMoa020057.

Brock-Utne, J.G., S.L. Gaffin, M.T. Wells, P. Gathiram, E. Sohar, M.F. James, D.F. Morrel, and. R.J. Norman. "Endotoxemia in exhausted runners after a long-distance race." *South Afr. Med. J.* 73 (1988): 533-536.

https://www.researchgate.net/publication/19780279_Endotoxaemia_in_exhausted_runners_after_a_long-distance_race

Chiossone, D. C., P.L. Simon, P.L. Smith. “Interleukin-1: effects on rabbit ileal mucosal ion transport in vitro.” *European Journal of Pharmacology* 180 no. 2-3 (1990): 217–228. DOI: 10.1016/0014-2999(90)90305-P.

Deopurkar R., H. Ghanim, J. Friedman, et al. “Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll-like receptor-4 and suppressor of cytokine signaling-3.” *Diabetes care* 33 no. 5 (2010):991–997.

Erridge, C., E. Bennett-Guerrero, and I.R. Poxton. “Structure and function of lipopolysaccharides.” *Microbes and Infection* 4 no. 8 (2002): 837-851. DOI: 10.1016/s1286-4579(02)01604-0

Fritsche, D. “Endotoxinpromovierte bakterielle Translokationen und Besiedelung von Uterus und Euter beim Hochleistungsrind im peripartalen Zeitraum.“ Dissertation. Leipzig, Univ., Veterinärmed. Fak. (1998)

Hanslin, K., J. Sjölin, P. Skorup, F. Wilske, R. Frithiof, A. Larsson, M. Castegren, E. Tano, and M. Lipcsey. “The impact of the systemic inflammatory response on hepatic bacterial elimination in experimental abdominal sepsis.” Intensive Care Medicine Experimental 7 (2019): art. 52. https://doi.org/10.1186/s40635-019-0266-x

Harte, A.L., N.F. da Silva, S.J. Creely, K.C. McGee, T. Billyard, E.M. Youssef-Elabd, G. Tripathi, E. Ashour, M.S. Abdalla, H.M. Sharada, A.I. Amin, A.D. Burt, S. Kumar, C.P. Day and P.G. McTernan. “Research Elevated endotoxin levels in non-alcoholic fatty liver disease.” *Journal of Inflammation* 7 (2010): 15-24. DOI: 10.1186/1476-9255-7-15

Hersoug, L.-G., P. Møller, and S. Loft. “Gut microbiota-derived lipopolysaccharide uptake and trafficking to adipose tissue: implications for inflammation and obesity.” Obesity Reviews 17 (2016): 297–312. DOI: 10.1111/obr.12370

Hurley, J. C. “Endotoxemia: Methods of detection and clinical correlates.” *Clin. Microbiol. Rev*. 8 (1995): 268–292. DOI: 10.1128/CMR.8.2.268

Kastner, A. “Untersuchungen zum Fettstoffwechsel und Endotoxin-Metabolismus bei Milchkühen vor dem Auftreten der Dislocatio abomasi.“ Inaug. Diss. Universität Leipzig, Veterinärmed. Fak. (2002). https://d-nb.info/967451647/34

Krüger M. “Escherichia coli: Problemkeim in der Nutztierhaltung.“ Darmflora in Symbiose und Pathogenität. Ökologische, physiologische und therapeutische Aspekte von Escherichia coli. 3. Interdisziplinäres Symposium. Alfred-Nissle-Gesellschaft (Ed.). Ansbach, 28.-29. Nov. (1997): 109-115.

Liang, Y.-C., H.-J. Liu, S.-H. Chen, C.-C. Chen, L.-S. Chou, and L. H. Tsai. “ Effect of lipopolysaccharide on diarrhea and gastrointestinal transit in mice: Roles of nitric oxide and prostaglandin E2.” *World J Gastroenterol.* 11 no. 3 (2005): 357–361. DOI: 10.3748/wjg.v11.i3.357

McAleer, J.P. and Vella, A.T. “Understanding how lipopolysaccharide impacts CD4 T cell immunity.” *Crit. Rev. Immunol.* 28 no. 4 (2008): 281-299. DOI:10.1615/CRITREVIMMUNOL.V28.I4.20

Morkok, A., L. Backstrom, L. Lund, A.R.Smith. “Bacterial endotoxin in blood of dysgalactic sows in relation to microbial status of uterus, milk, and intestine.” JAVMA 183 (1983): 786-789. PMID: 6629987

Munck, L.K., A. Mertz-Nielsen, H. Westh, K. Buxhave, E. Beubler, J. Rask-Madsen. “Prostaglandin E2 is a mediator of 5-hydroxytryptamine induced water and electrolyte secretion in the human jejunum.” *Gut* 29 no. 10 (1988): 1337-1341

Pig Progress. “Mastitis, Metritis, Agalactia (MMA).” https://www.pigprogress.net/Health/Health-Tool/diseases/Mastitis-metritis-agalactia-MMA/

Sampath, V.P. “Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates.” *Agriculture and Natural Resources* 52 no. 2 (2018): 115-120. https://doi.org/10.1016/j.anres.2018.08.002

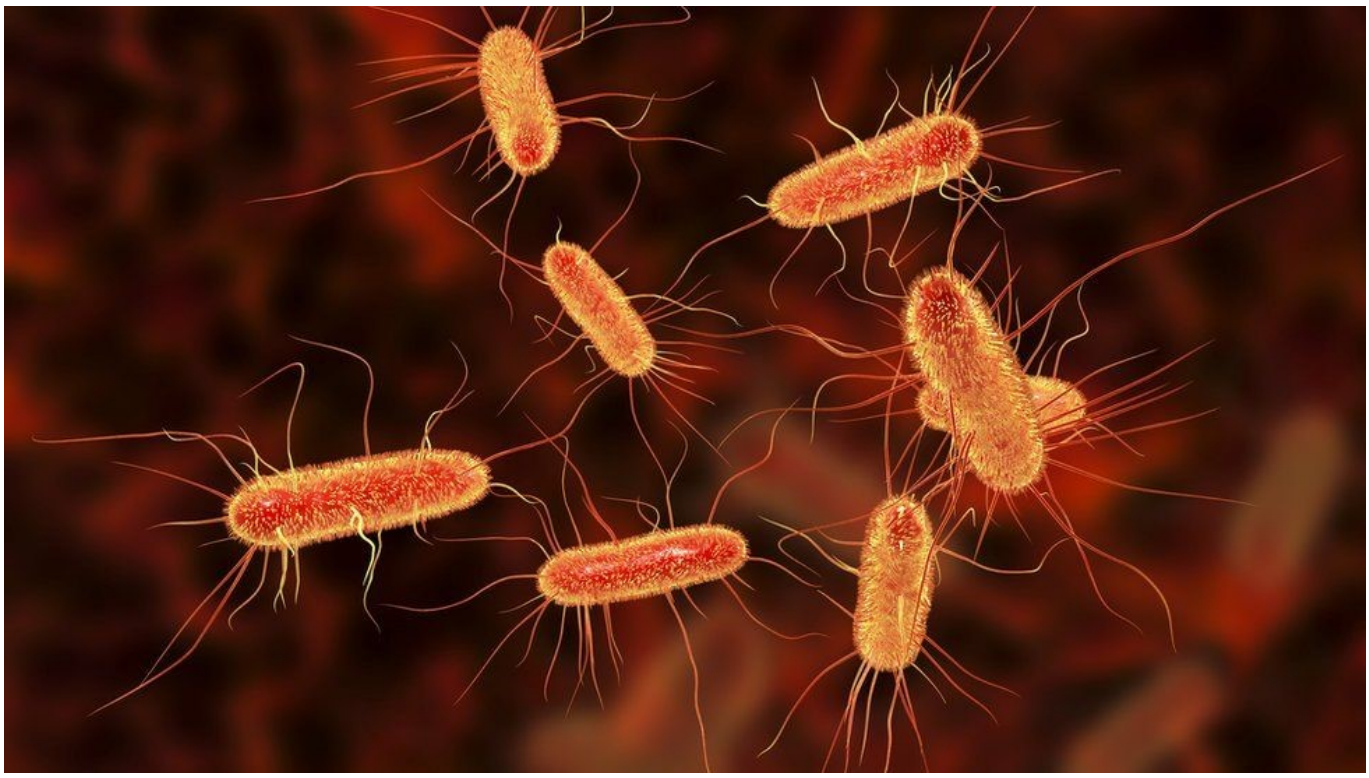
Seidler, T. “Freies Endotoxin in der Blutzirkulation von Schlachtschweinen: eine Ursache für bakterielle

Translokationen?“ Diss. Universität Leipzig, Veterinärmed. Fak. (1998).

Smith, B.B. and W.C. Wagner. “Suppression of prolactin in pigs by Escherichia coli endotoxin.“ Science 224 no. 4649 (1984): 605-607

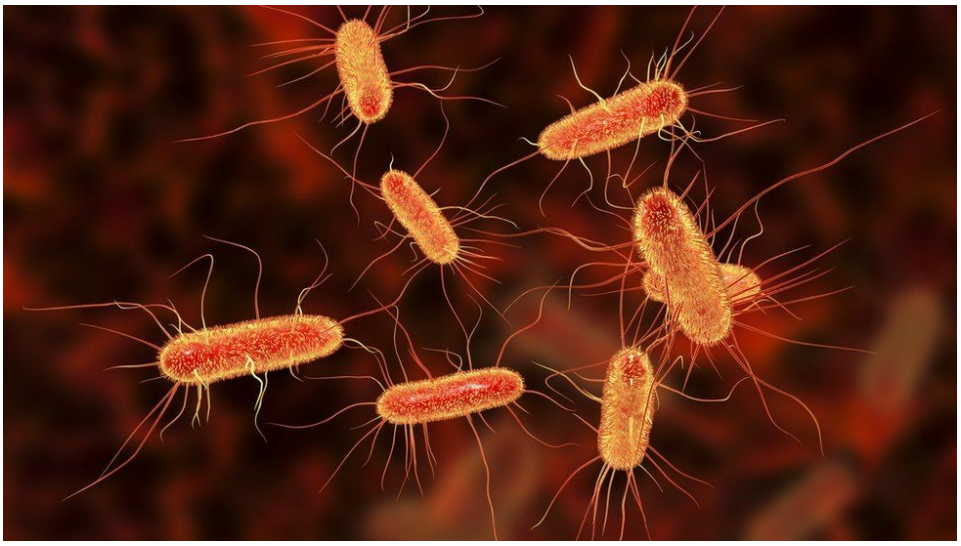
Wilken, H. “Endotoxin-Status und antioxidative Kapazität sowie ausgewählte Stoffwechselparameter bei gesunden Milch- und Mutterkühen.“ Inaugural Diss. Universität Leipzig (2003).

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).

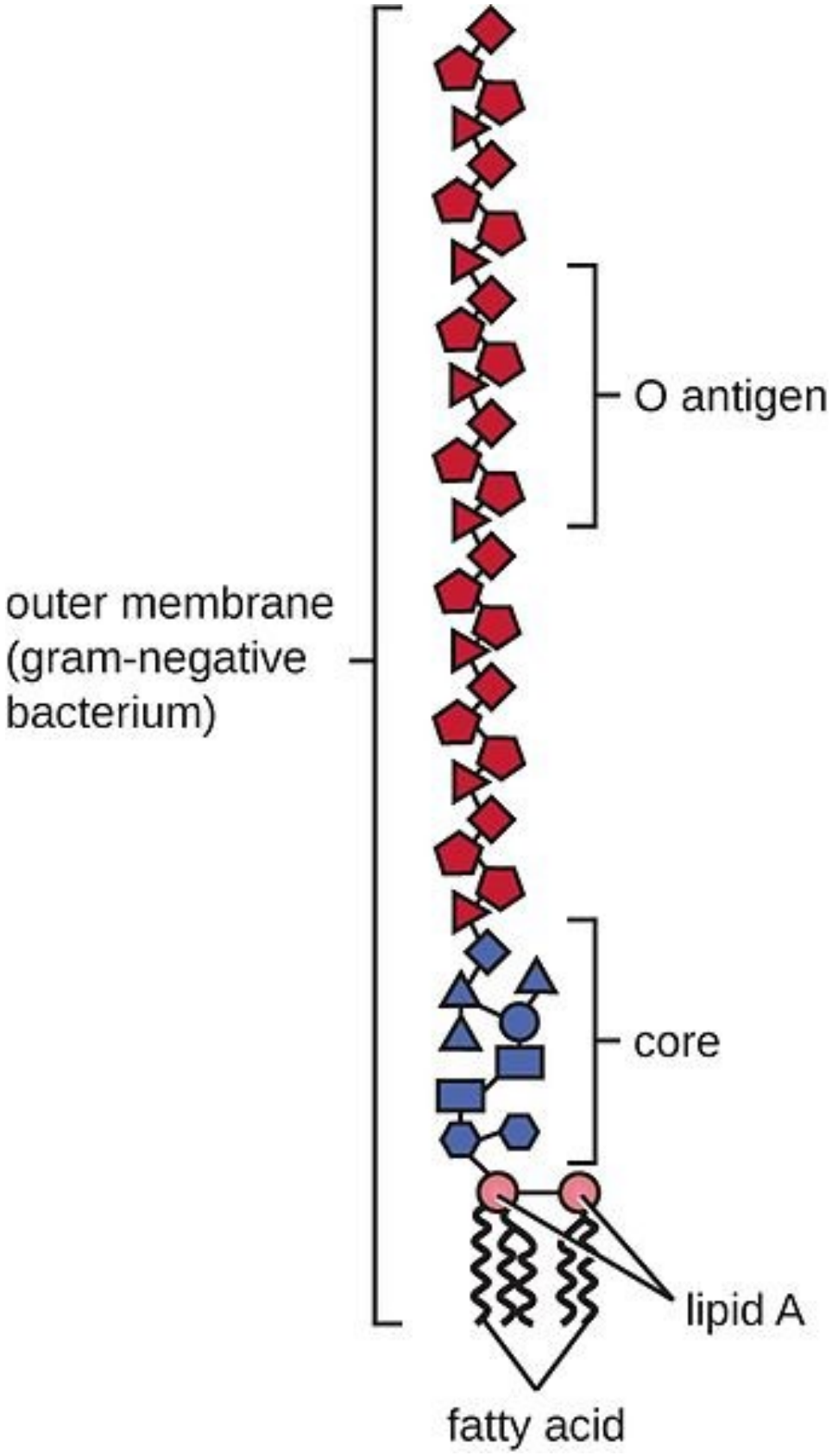


Figure 1: Structure of an LPS

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).

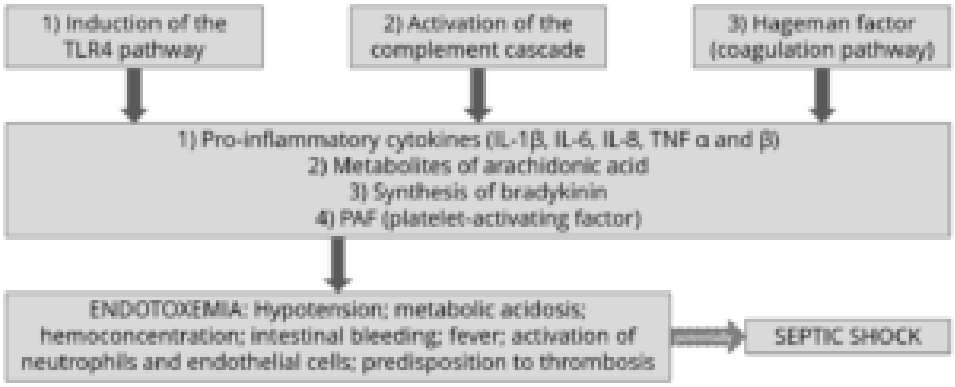


Figure 2: How LPS leads to endotoxemia – 3 modes of action

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.

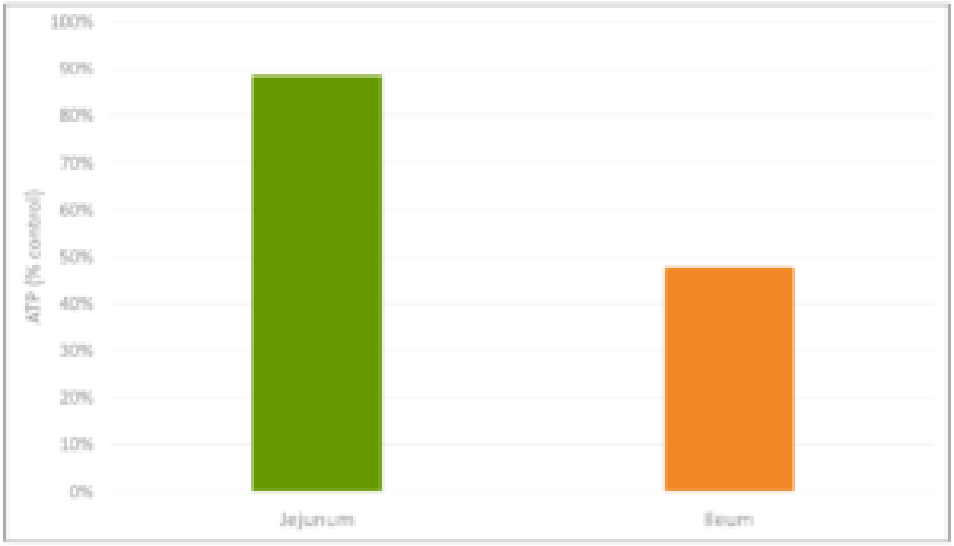


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from Li et al., 2015)

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.

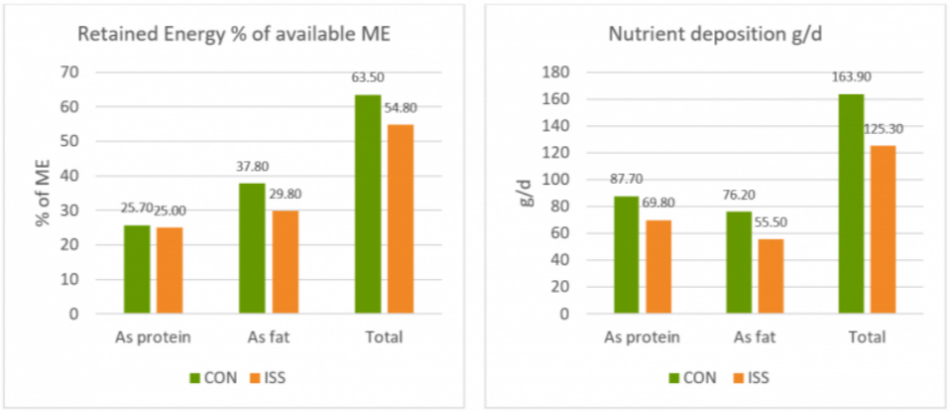


Figure 4: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from Huntley, Nyachoti, and Patience, 2017)

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.

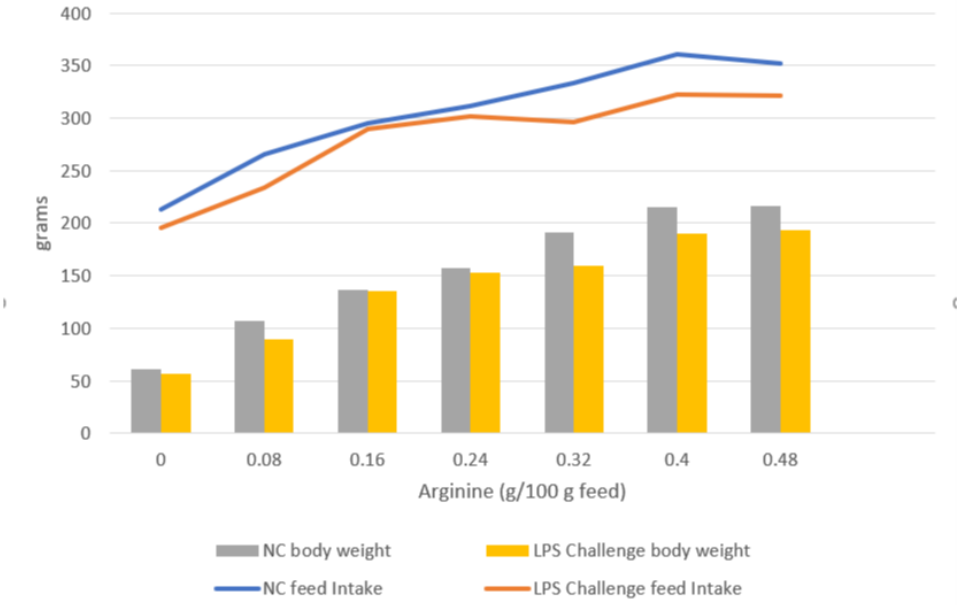


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on Webel, Johnson, and Baker, 1998)

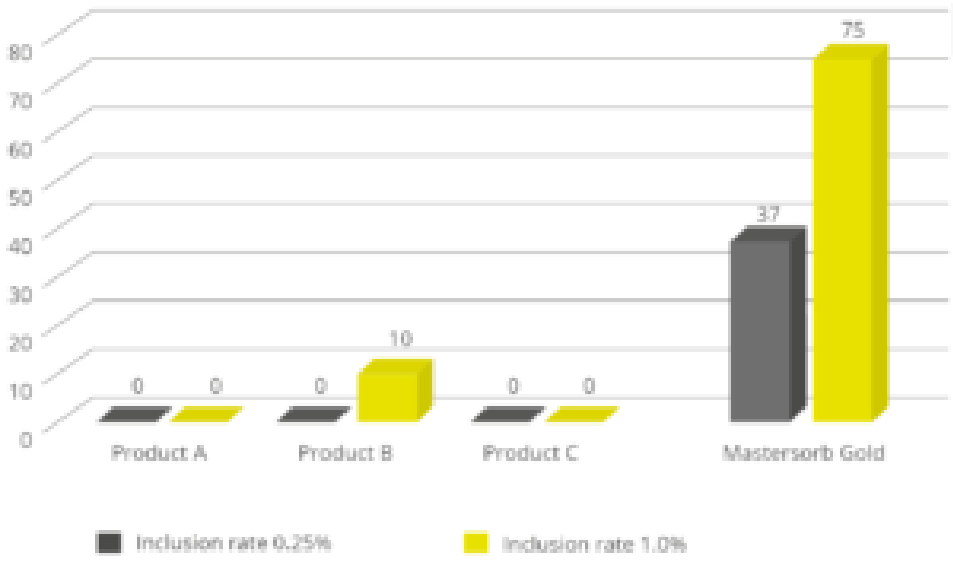
NC = negative control

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.

By Claudio Campanelli, EW Nutrition

References

Adib-Conquy, Minou, and Jean-Marc Cavaillon. "Compensatory Anti-Inflammatory Response Syndrome." *Thrombosis and Haemostasis* 101, no. 01 (2009): 36–47. https://doi.org/10.1160/th08-07-0421.

Huntley, Nichole F., C. Martin Nyachoti, and John F. Patience. "Immune System Stimulation Increases Nursery Pig Maintenance Energy Requirements." *Iowa State University Animal Industry Report* 14, no. 1 (2017). https://doi.org/10.31274/ans_air-180814-344.

Li, Jiaolong, Yongqing Hou, Dan Yi, Jun Zhang, Lei Wang, Hongyi Qiu, Binying Ding, and Joshua Gong. "Effects of Tributyrin on Intestinal Energy Status, Antioxidative Capacity and Immune Response to Lipopolysaccharide Challenge in Broilers." *Asian-Australasian Journal of Animal Sciences* 28, no. 12 (2015): 1784–93. https://doi.org/10.5713/ajas.15.0286.

Mani, Venkatesh, James H Hollis, and Nicholas K Gabler. "Dietary Oil Composition Differentially Modulates Intestinal Endotoxin Transport and Postprandial Endotoxemia." *Nutrition & Metabolism* 10, no. 1 (2013): 6. https://doi.org/10.1186/1743-7075-10-6.

Webel, D.M., R.W. Johnson, and D.H. Baker. "Lipopolysaccharide-Induced Reductions in Body Weight Gain and Feed Intake Do Not Reduce the Efficiency of Arginine Utilization for Whole-Body Protein Accretion in the Chick." *Poultry Science* 77, no. 12 (1998): 1893–98. https://doi.org/10.1093/ps/77.12.1893.

Zachary, James F. "Chapter 4 – Mechanisms of Microbial Infections." Essay. In *Pathologic Basis of Veterinary Disease*, 132–241. St Louis, MO: Mosby, 2017. https://doi.org/10.1016/B978-0-323-35775-3.00004-7.