How to reduce methane emissions in dairy cows: phytogenic solutions

by Technical Team, EW Nutrition

The world demand for milk has seen a sharp rise. Today, we have just over 1 billion dairy cows in the world producing about 1.6 billion tons of milk per year. However, OECD and FAO estimate that numbers will rise up to 1.5 billion dairy cows in 2028, for a total milk production of 2 billion tons . This increase will come at a tremendous cost in terms of global warming: Each day, dairy cows can produce 250 to 500 litres of methane, a powerful greenhouse gas (Johnson and Johnson, 1995).

Climate change is not the only reason for zootechnical production to adopt methane reduction strategies. Methane emissions represent an important energy loss for dairy cows, which negatively impacts production performance. In this article, we review why methanogenesis in dairy cows arises, and how the use of phytogenic product Activo Premium can help achieve efficient energy use and reduced climate impact.

Less methane: environmental, regulatory, and business pressures

Methane (CH_4) is considered one of the gases that, together with CO_2 (carbon dioxide) and N_2O (nitrous oxide), traps heat in the atmosphere and, thus, causes global warming. While methane is generated in multiple industries, including the energy and waste sectors, much of the methane present in the atmosphere derives from livestock activities and, in particular, from ruminant farms.

About 28% of total methane emissions derive from agriculture sector and enteric fermentations (digestive processes in which feed is broken down by microorganisms) are responsible for about 65% of the total methane coming from zootechnical sector (Knapp et al., 2014). For this reason, in recent years, strategies for mitigating methane emissions in dairy cows have aroused great interest among researchers and environmentally-conscious consumers.

Regulators have also caught on: In October 2020, the European Commission presented its strategy for reducing methane emissions in Europe. Reductions are essential to achieve the Commission's climate objectives for 2030 and climate neutrality by 2050. For the livestock sector, the Commission seeks to develop an inventory of innovative mitigating practices by the end of 2021, with a special focus on methane from enteric fermentation.

Uptake of mitigation technologies will be promoted though Member States' and the Common Agricultural Policy's "carbon farming" measures. Carbon-balance calculations at farm level are to be encouraged through digital tools; and the Horizon Europe strategic plan 2021-2024 will likely include targeted research on effective reduction strategies, focusing on technology, dietary factors, and nature-based solutions such as phytogenic products.

Even aside from environmental concerns, consumers demands, and regulatory steps, there is a critical business case for dairy producers to lower methane emissions. Given the ever-increasing global demand for dairy products, farmers and other operators in the sector more than ever try to maintain and indeed improve production to maximize yields, both economically and in terms of finished products. Problematically, methane production in the rumen represents a great loss of energy for the animal.

On average, about 6% of the total energy ingested by a dairy cow is transformed into methane, every single day (Succi and Hoffmann, 1993). The less methane a cow produces, the more metabolizable energy (ME) she gets out of her gross energy (GE) intake. A better ME/GE ratio translates into higher net energy of lactation (NEl). Energy losses from methanogenesis thus directly decrease the energy nutritionist can consider as usable during rationing.

Before we review the current research on how an adequate manipulation of the diet and of the rumen environment can mitigate these energy losses, we need to ask ourselves, why is methane formed in the rumen at all?

Animal physiology: how methane is formed in the rumen

Ruminants' digestion of vegetal ingredients is linked to their rumen's symbiotic bacterial, protozoan, and fungal flora. This microbiota has all the enzymatic properties necessary for the digestion (or rather pre-digestion) of ingested forage, including some cellulose fractions that monogastric animals cannot use.

In the rumen, the main products deriving from bacterial fermentation are volatile fatty acids and methane. The main volatile fatty acids are acetic acid, propionic and butyric acid, which are mainly absorbed and used by the animal. Meanwhile, methane helps to maintain the oxidative conditions in the rumen' anaerobic environment, but also represents an energy loss (Czerkawski, 1988).

Methanogenesis is carried out by methanogenic bacteria and *archae* in the rumen (Guglielmelli, 2009). They use molecular hydrogen and carbon dioxide as a substrate for the synthesis of methane, according to the following equation:

$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$

A few other chemical reactions contribute to methanogenesis, but they all have one thing in common: they

require hydrogen ions in the rumen fluid to form methane from CO_2. This gives us the first “point of attack” for reducing methane formation: the diet.

Increase the share of propionic acid

Propionic acid is in competition with methanogens in using hydrogen ions to reduce glucose molecules:

$C_6H_{12}O_6$ (glucose) + 4 H → 2 $C_3H_6O_2$ (propionic) + 2 H_2O

It is clear that if propionic fermentations are stimulated through the diet at the expense of the pathways leading to acetate and butyrate (where hydrogen ions are transferred to the rumen environment), the availability of hydrogen for the reduction of CO_2 by methanogenic bacteria decreases.

Diets with a high level of concentrates, and low levels of neutral detergent fibre, yield more propionic acid and less acetic and butyric acid. Set aside lower methane emissions, this increase in energy is desirable during peak lactation: the energy gap that follows from the decrease in ingestion by the animal requires diets with a high amount of substrate for gluconeogenesis. Furthermore, the greater production of propionate sequesters H_2 in the rumen environment and, consequently, less CO_2 is reduced to methane.

Optimize the protozoa count

Most methane-producing bacteria live in symbiosis with most of the protozoan species, they are located on the surface of the protozoan. It follows that optimizing the population of protozoa present in the rumen (through dietary measures) leads to a lower methanogenesis (Patra and Saxena, 2010). Naturally, a minimum amount of protozoa must be maintained to avoid excessively reducing ruminal motility (regular contractions that mix and move the rumen content), which is important for feed digestibility.

Diet is not enough: feed additives to reduce methane production

Dietary measures alone cannot considerably reduce daily methane production. In the past, antibiotic growth promoters belonging to the ionophores family were commonly administered in the EU. These antibiotics increase efficiency and daily weight gain by promoting gluconeogenesis through greater production of propionic acid in the rumen and a consequent reduction in emitted methane (Piva et al., 2014).

The emergence of bacterial forms resistant to growth-promoting antibiotics have forced the EU to ban these molecules to safeguard consumer health. Fortunately, certain feed additives can also help reduce methanogenesis and generate energy saving – without the danger of resistance.

Secondary plant extracts or phytomolecules feature relevant properties, including bactericidal, virucide, and fungicide effects. As we have seen, it is critical to encourage certain fermentations at the expense of others and possibly reduce the organisms directly and indirectly responsible (bacteria and protozoa) for methanogenic fermentations.

Activo Premium: reduce methane and

preserve energy

Phytogenic product Activo Premium contains a targeted phytomolecules mix capable of influencing the rumen microbiome in this manner:

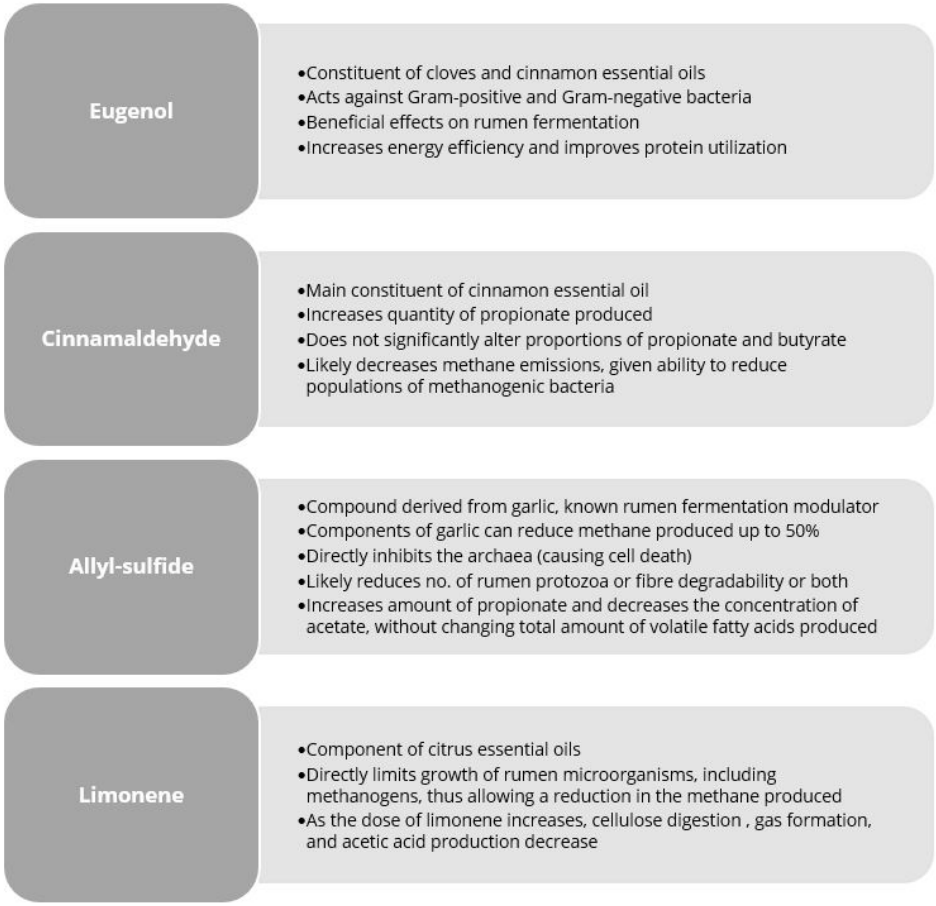

Figure 1: Anti-methanogenic properties of selected phytomolecules. Based on Lourenço et al. (2008) and Supapong et al. (2017)

Activo Premium is a blend of phytomolecules that maximizes production results for both high- and low-energy diets. Studies show that Activo Premium's effects on the on the rumen microbiome reduce the ratio of acetic to propionic and butyric acid and decrease the energy losses due to methane production.

Field trial: Activo Premium improves rumen fermentation processes

A trial at the University of São Paul, Brazil, sought to evaluate the impact of Activo Premium on rumen fermentation and methane emissions. Nine rumen-cannulated sheep (55 ± 3.7 kg of body weight) were divided into 3 groups, and randomly distributed in a triple 3×3 Latin square design. The animals were fed their experimental diets for 22 days (the sampling period) in the following 3 set-ups: one control group (basal diet without additives); one group receiving a basal diet with 200 mg of Activo Premium per kg of dry matter intake; and one group receiving a basal diet with 400 mg of Activo Premium per kg of dry matter intake.

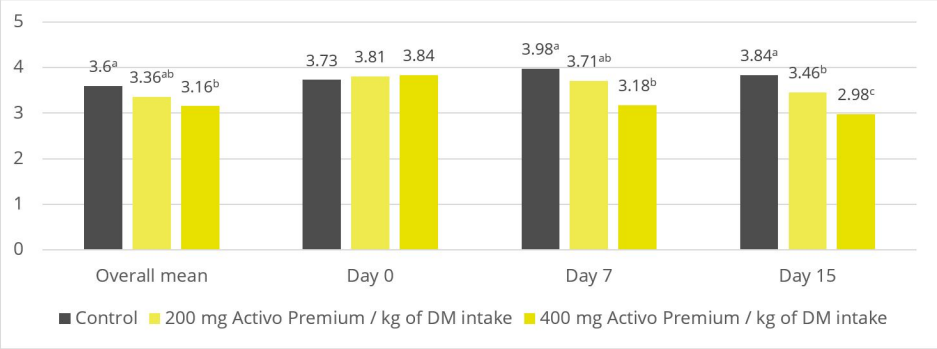

Figure 2: Ratio of acetate to propionate (p = 0.03)

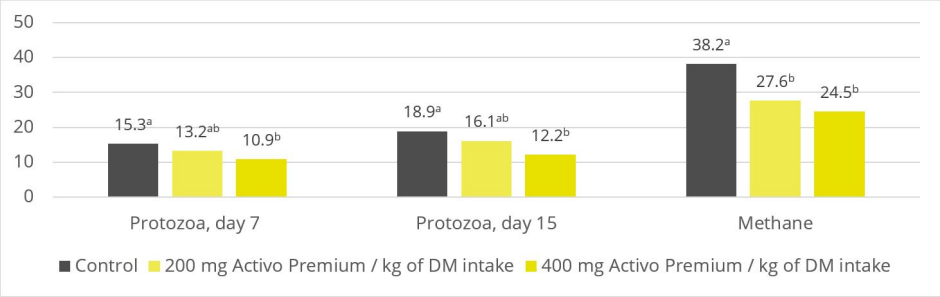

Figure 3: Protozoa count (p = 0.06; x 10^5 / ml) and methane production (p < 0.01; l per kg of dry matter). Based on Soltan et al. (2018)

As shown in figures 2 and 3, Activo Premium favourably modifies the ratio of volatile fatty acids and reduces the protozoa count, which, as to be expected, results in reduced methane emissions.

Rumen simulation trial: the more Activo Premium added, the less methane produced

A trial was conducted at the University of Hohenheim (Germany) sought to evaluate the methane-reducing effects of different inclusion rates of Activo Premium, using a continuous long-term rumen simulation technique (Rusitec). Four different inclusion levels of Activo Premium (0, 2.1, 4.2, and 8.4 mg/d) were added to a diet with a ratio of concentrates to roughages of 80% to 20%, respectively.

Five consecutive Rusitec runs with one replication of each of the four inclusion schedules were performed. The run lasted for 14 days; 7 days were used for adaptation and the later 7 days for sampling. The fermenters were heated to 39°C. During the sampling period, total gas production and methane concentration of the total gas produced were measured every 24 h.

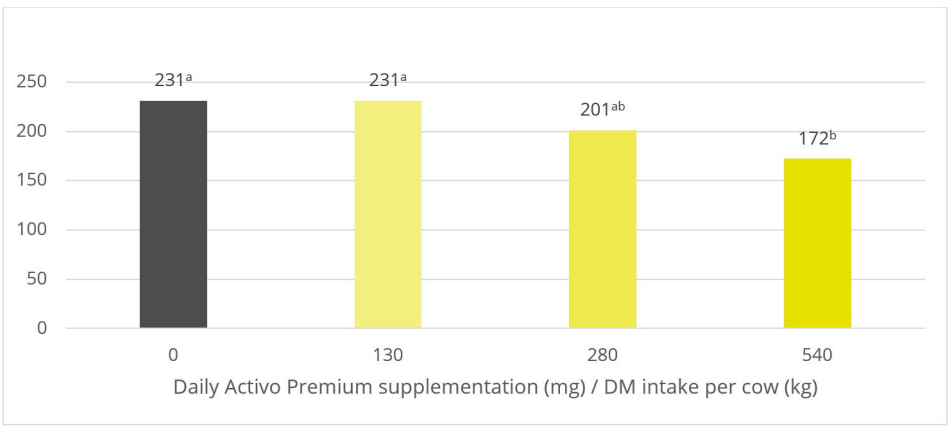

Figure 4: Methane emission (ml / day) for increasing inclusion rates of Activo Premium

In this trial with a rumen simulation system, Activo Premium significantly reduced methane volume (Figure 4): from 231 ml/d for the diet without any Activo Premium to 172 ml/d for the highest inclusion rate of Activo Premium.

Activo Premium: reduce methane emissions, support your profits and our planet

Both *in vivo* and *in vitro* trials have shown with high statistical reliability that Activo Premium can positively modulate rumen fermentations. The strategic combination of phytomolecules appears highly effective as a natural dietary supplementation option to modulate ruminal fermentation and decrease methane emissions. Adding Activo Premium to dairy cows' diet will likely contribute significantly to reducing their methane emissions and optimizing their energy balance – improving animal performance while curbing the climate change impact, a win-win for everyone.

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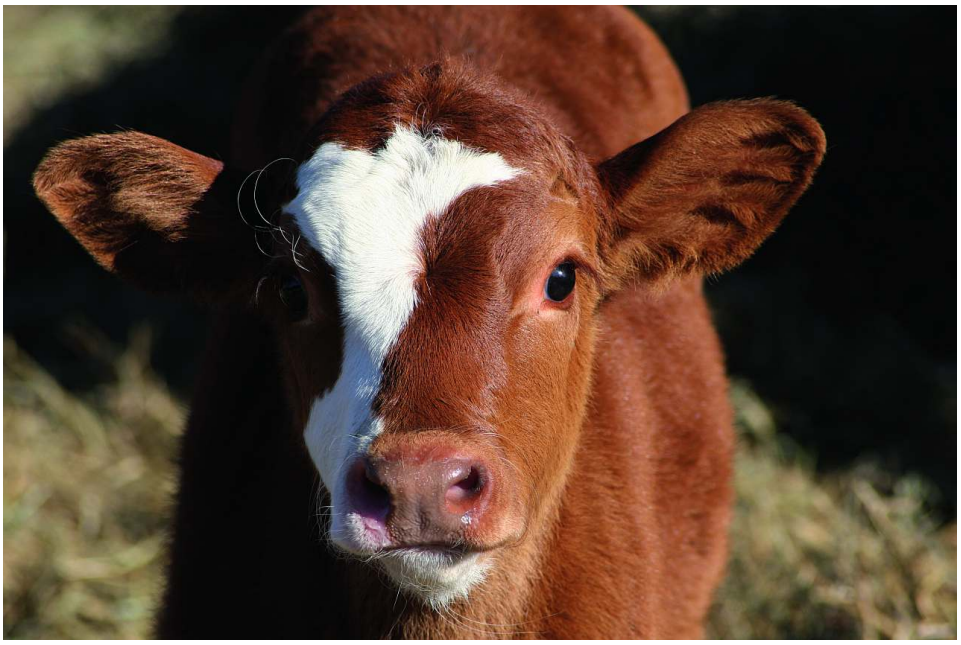
Diarrhea in calves: Causes, consequences, prevention

by Judith Schmidt, ProductManager On Farm Solutions, EW Nutrition

Although diarrhea is called a factor disease, strictly speaking, it is not a disease but a symptom. Diarrhea can be a protective function of the body. With the higher fluid volume in the intestine and its increased peristalsis, pathogens and toxins are excreted.



Common causes of diarrhea

Despite various electrolyte drinks available from the veterinarian or in stores, too many calves still die as a consequence of diarrhea. The economic damage for the farms is immense.

The causes of the occurrence of diarrhea are diverse. Infectious causes such as viruses, parasites, bacteria, fungi, and non-infectious causes such as insufficient colostrum supply, feeding, and housing have a significant influence.

The diet of the newborn calf has a significant influence on scours. The following factors are decisive:

- The immune status of the calves
- Inadequate/incorrect preparation of the liquids
- Inadequate drinking hygiene

The development of diarrhea

In the first three weeks of life, diarrheal diseases are the most common and economically impactful diseases in newborn calves. In the first weeks of life, 75 to 85 % of calf diseases are related to diarrhea. The reason for this is that calves are born without immune protection. Their immunity is primarily built up in the first twelve hours by the supply of colostrum. After that, the intestinal barrier is barely passable for the antibodies.

The four most important pathogens are Rotavirus and Coronavirus, Cryptosporidium, and E.coli. These pathogens damage the intestinal lining, leading to water and minerals not being absorbed from the intestine into the blood. The minerals, instead of being assimilated, are lost and eliminated through feces.

Bacteria such as E.coli attach to the intestinal wall and produce toxins. Viruses, on the other hand, penetrate the intestinal wall in order to multiply. Both of them result in damages to the intestinal wall, which can allow fluids to leak out. The result is diarrhea.

Symptoms of diarrhea

The most important symptoms are:

- Sunken eyes as an expression of dehydration
- Reduced intake of fluids
- Lying down
- Low temperature
- Cold body surface
- Apathy or even coma

Types of diarrhea

There are different types of diarrhea, mainly the secretory and the malabsorptive form. Because of frequent mixed infections, the two forms of scours are often mixed.

Secretory diarrhea

The binding of toxins to the enterocytes' cell surface receptors activates enzyme systems that lead to increased fluid secretion in the intestine. The intestinal lining can no longer absorb this increased fluid influx. The trigger for this can be, for example, an E.coli infection.

Malabsorptive diarrhea

The erythrocytes are destroyed and the villi are reduced in size. There is a loss of the microvilli. The result is a lower enzyme activity and resorption capacity. By this reduction in villi length, less fluid can be absorbed and has to be excreted through the intestine.

Importance of the colostrum supply

Low colostrum intake or a low quality of colostrum at birth results in the failure of passive transfer (FPT) due to the inadequate ingestion of colostral immunoglobulins. FPT is associated with an increased risk of mortality and decreased health status.

Adequate transfer of maternal immunoglobulins is associated with short- and long-term health advantages. These advantages are created by reducing pre- and post-weaning mortality due to infectious diseases, as well as by increasing daily weight gain, feed efficiency, fertility, and milk production in first and second lactation.

Colostrum is the elixir of life for newborn calves. As already mentioned, calves are born without their own active immune protection. Their immune system develops slowly. In order to obtain a first passive immunization, early administration of high-quality colostrum (≥ 50 mg IgG/ml) is of the highest importance.

The colostrum should be administered to calves as early as possible, but latest 4 to 6 hours after birth. The reason for early administration of colostrum is that the amount of immunoglobulins decreases with the passage of time after birth and with an increased milking number.

By the twelfth week, the calf has fully developed its own stable immune system and is therefore able to produce its own antibodies.

Economic consequences of diarrhea

The consequences of diarrhea and the associated costs should not be underestimated. Even a mild form of diarrhea costs the farmer money:

	Course of diarrhea			
	Heavy diarrhea		Light diarrhea	
	In €	In %	In €	In %
Costs for Vet Costs for drugs, electrolytes	75 € 72 €	56	45 € 30 €	69
Additional rearing days Additional rearing costs	9 days 30,60 €	12	4 days 13,60 €	13
Mortality rate Costs for mortality	13 % 48 €	18	2 % 7,40 €	7
Additional labor farmer Additional costs for labour	2,5 h 37,50 €	14	0,8 h 12 €	11
Overall costs	**263,10 €**		**108,00 €**	

How to avoid diarrhea in calves

It is primarily essential that the calf is protected from fluid losses and that active diarrhea is avoided. Measures can be taken in advance to prevent the newborn calves from diarrhea:

- Cleaning the calving pen after each calving
- Bringing the calves into cleaned and disinfected boxes
- Regularly checking the quality of colostrum

But the most important basic requirement for a healthy start into life is to give 2 to 4 liters of colostrum within the first six hours of life. In addition to the timing, the quality of the colostrum is crucial. To that end, EW Nutrition developed a colostrum enhancer that improves colostrum management.

IgY can bind foreign substances like bacteria or viruses in the gut, which improves gut health and increases weight gain. The natural egg immunoglobulins act like maternal colostrum and bind to the pathogen epitopes. After that, the blocked pathogens cannot bind to the intestinal wall, preventing damage to the intestinal wall. Field studies prove the product's efficacy, showing an 18 % higher daily weight gain and a 13 % higher weaning weight compared to the control group. Additionally, the IgG contained in Globigen Colostrum help you avoid a failure of passive transfer (FPT).

The application of Globigen Colostrum is very user-friendly and simple, as it can be mixed directly into the colostrum of the mother cow.

Higher profit through improved calf performance

The benefits of Globigen Colostrum are:

- Improved calf performance
- Lower incidence of diarrhea
- Improved weight gain
- Higher profit

The timely and adequate supply of colostrum is the most important factor in preventing infection-related calf diseases. Therefore, it is necessary to ensure that calves receive sufficient antibodies from the cow's colostrum in the first days after birth.

References available upon request

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).

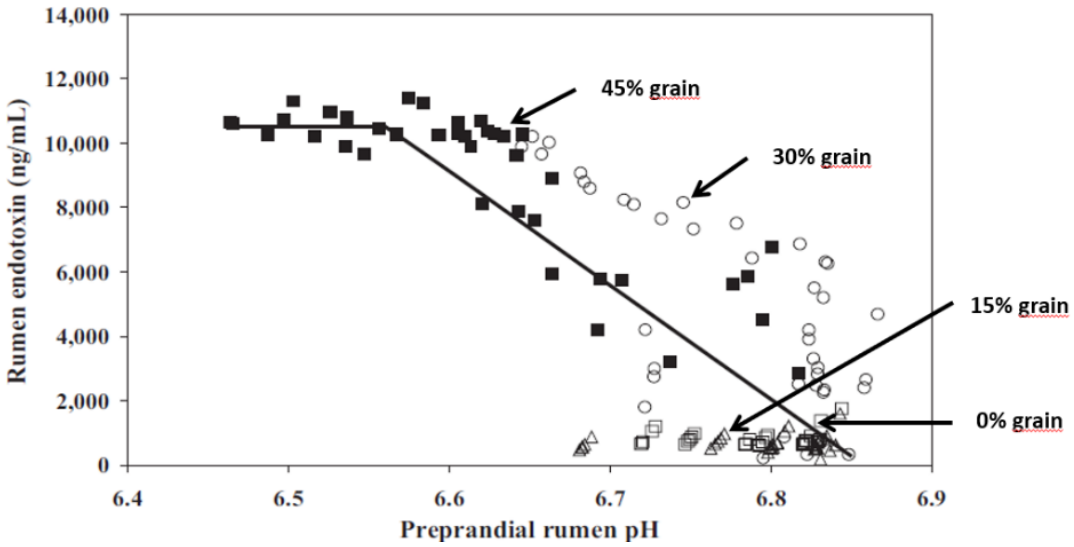


Figure 1. The increase in the level of endotoxins in the rumen is directly correlated with an increase in ration concentrates

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.

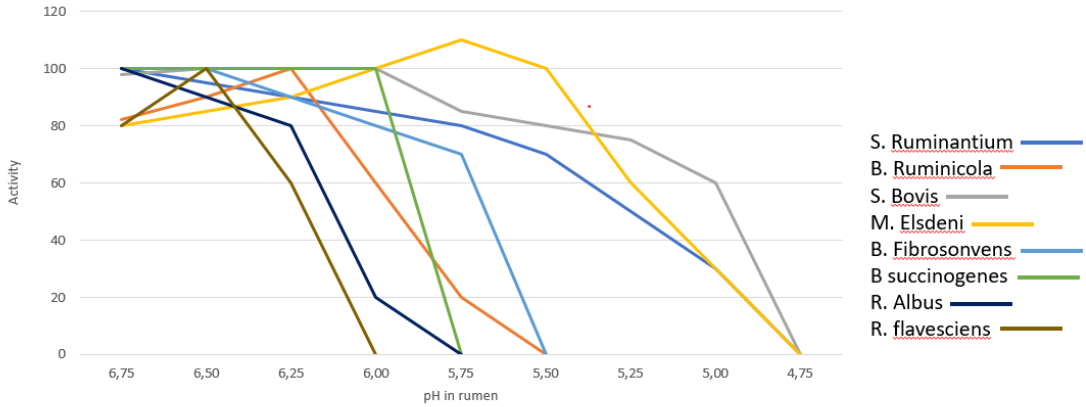


Figure 2. Activity of main bacteria in the rumen in function of pH (Daniele Cevolani Edizioni Agricole di New Business Media srl 2020)

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).

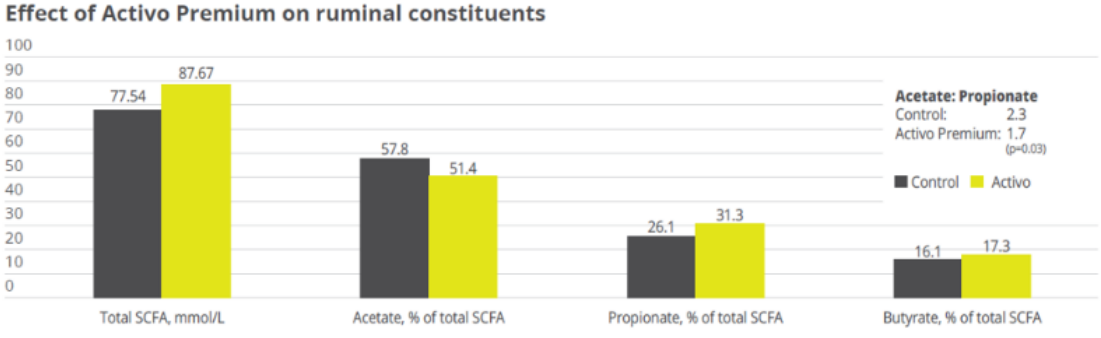


Figure 3. Effect of Activo Premium on ruminal constituents

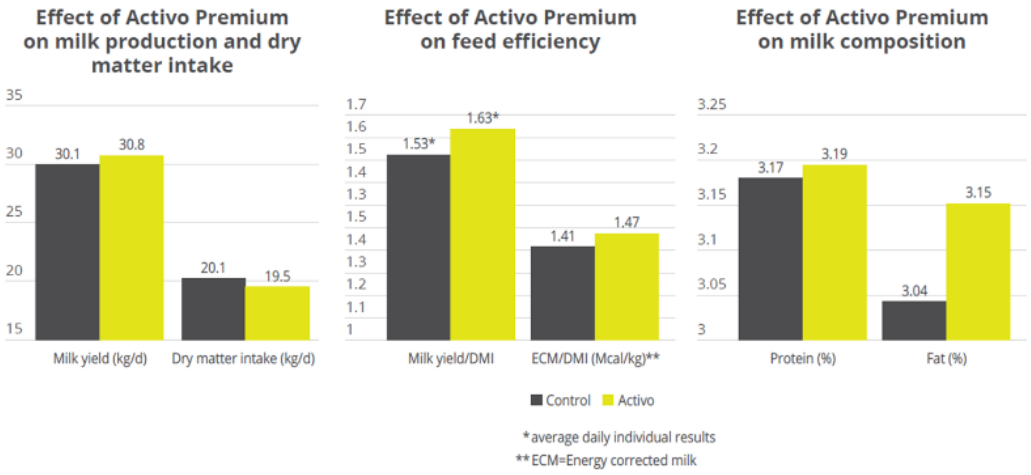


Figure 4. Effect of Activo Premium on animal performance

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.

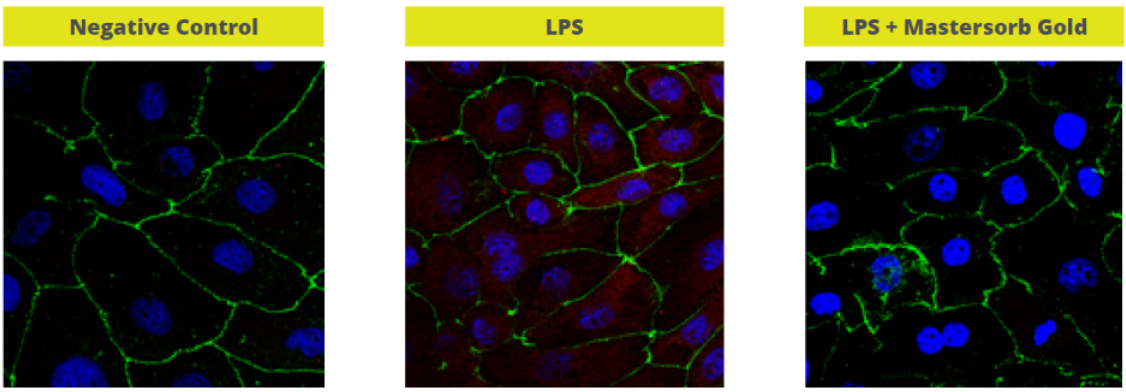


Figure 5. Immunofluorescence in PEG-J2: Challenge with LPS without (in the middle) and with Mastersorb Gold (right)

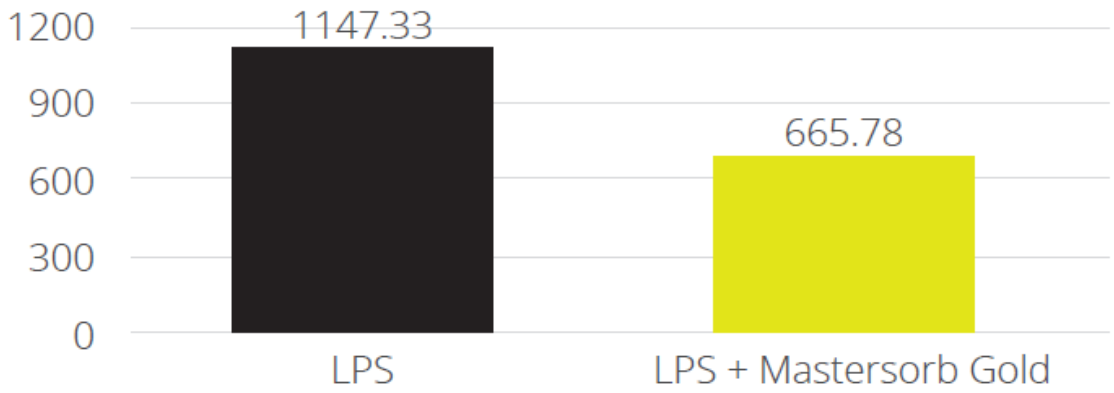


Figure 6. IL-8 AP secretion after incubation with LPS 0111:B4 for 24h without and with Mastersorb Gold

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.

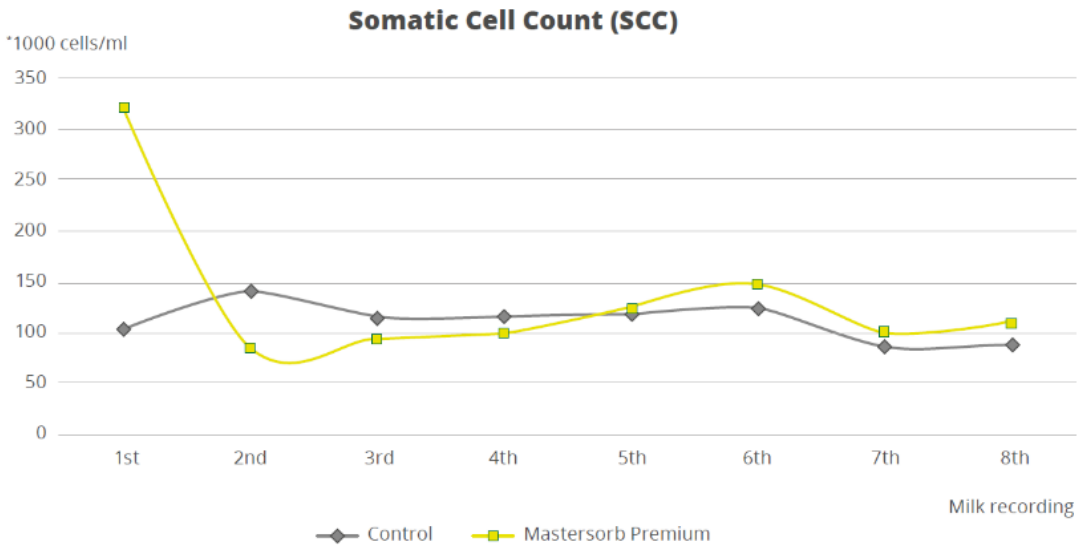


Figure 7. Effect of Mastersorb Premium on somatic cell count

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.

Milk fever: Causes, consequences, prevention

Find out more about On Farm Solutions here

Nowadays, dairy cows are real top athletes. This comes with additional challenges for their health and for on-farm management. Many of these problems can be traced back to supply deficits and can be easily managed with appropriate feed supplements.

Milk fever is a disease that occurs mainly in cows around calving. It is caused by an insufficient amount of calcium in the blood and particularly affects cows with a very high milk yield.

The link between calcium and milk fever

Calcium performs essential functions in the body. It is particularly important for the nervous system and muscle cells, and plays a central role in muscle contraction. If the calcium content in the blood is too low, the muscles can no longer contract. When this happens, the cows cannot move or stand up.

While mild cases may not be easily detectable, they still trigger productivity loss. If undetected, long-term calcium deficiency can even lead to cardiac arrest and thus to the death of the animal.

The development of milk fever

The cause of milk fever is a lack of sufficient calcium in the blood serum (hypocalcemia). The dairy cow has to abruptly change its metabolism at the end of the dry period, going from the resting phase to a high performance phase. During the dry period, cows have a relatively low need for calcium.

When lactation starts, the need for calcium suddenly almost doubles, as large amounts of calcium are required for the production of colostrum (2.3 g/l). The calcium is generally drawn from feed or from the bones. In older cows, the mobilization mechanism often does not start quickly enough. The supply from the bones and feed is insufficient and the body draws the missing calcium from the muscles. This ultimately leads to symptoms of paralysis and overstimulation of the nervous system.

Phases of milk fever

Stage One

In the initial phase of milk fever, the initial signs are

- muscle tremors
- restlessness
- stiff gait
- slightly elevated temperature

Stage Two

At this point, the cows lie on the stomach with an extended neck or the head is lying on the flank. Early symptoms of paralysis appear:

- fast, flat pulse
- cold body surface
- dilated pupils
- flatulence

Stage Three

In the last phase of milk fever, the cow lies on its side, loses consciousness and falls into a coma. The third phase often leads to death (the mortality rate averages 2 – 5%).

While the second phase of milk fever is easy to recognize due to the clear symptoms, the consequences of a “slight” calcium deficiency (Stage One) are often underestimated. Feed intake diminishes, the negative energy and protein balance is increased, and the cows barely move. The impairment of the muscles can cause problems in the udder (mastitis) or in the gastrointestinal tract.

Prevention and solutions

As cases of hypocalcemia immediately after calving may be as high as 50% among second- or third-lactation cows, it is important to act preventively to keep potential milk fever from developing. The dairy farmer´s aim is to support the dairy cows that are at higher risk of milk fever, especially around the critical time of calving. The cows must be enabled to quickly release calcium from the bones after calving, or they must be supplied with calcium that can be easily metabolized.

Upfront prophylaxis

An energy and protein oversupply during the dry period should be avoided. In addition, an application of Vitamin D3 at the end of the pregnancy makes sense.

To stimulate the active regulatory mechanisms of calcium metabolism, the calcium content in the feed should be reduced three to four weeks before calving. In practice, however, this often is not properly observed and feed with a relatively high calcium content is still given out during this period.

There are, no doubt, farms where these above-mentioned preventive measures cannot be carried out due to operational reasons, just as there are animals that are particularly susceptible due to factors such as age, breed or healthy history.

To protect the cow from milk fever around calving, oral administration of calcium salts is widespread in practice. Vitamin D also plays a central role in calcium metabolism. It ensures that the absorption of

calcium from the intestines and bones is increased.

When administering oral calcium supplements, there are three important points:

– The cow must have sufficient calcium available per dosage

– The calcium must be available immediately

– Administration must be appropriate for the animals and farmers

Methods of calcium supplementation

To support the cow, oral supplements such as pastes and gels are widely used. They are useful, however they are also relatively difficult to administer, as they require handling the animal in relatively difficult ways.

Liquids are another way of administering calcium supplements. When administering liquids, it is important to make sure the animal does not choke so that the liquids do not get into the lungs.

Boluses are probably the easiest and safest method of supplementation to prevent milk fever. The bolus must naturally be carefully inserted, however the process is easy and requires minimal handling of the animal.

EW Nutrition´s Calzogol Bolus is a dietetic mineral feed with a high level of calcium from of highly available calcium salts and vitamin D3. The Calzogol Bolus contains several calcium sources with different release rates. One major advantage is the very high mucous membrane compatibility, which helps avoid irritation of the mouth, esophagus and rumen. Furthermore, the Calzogol Bolus does not contain caustic calcium chloride. The application is simple and economical, as only one bolus per dose must be administered at the time of calving.

Conclusion

Milk fever is very common in dairy herds. When a cow has milk fever, the farm can incur costs of approx. €350. This is reflected in the loss of milk yield up to 600 kg, losses due to unusable milk, and veterinary and medication costs.

Time resources are also to be taken into account: The economic repercussions represent a significant factor, however they come on top of the extra workload due to the increased need for care of animals.

Cows that suffer from calcium deficiency are also much more susceptible to other diseases. For the farmer, the best strategy is to avoid losses through prophylaxis. Feeding plays a central role; to ensure the best possible production conditions, oral calcium administrations, such as Calzogol Bolus, have proven themselves in practice.

by Judith Schmidt, Product Manager, On Farm Solutions

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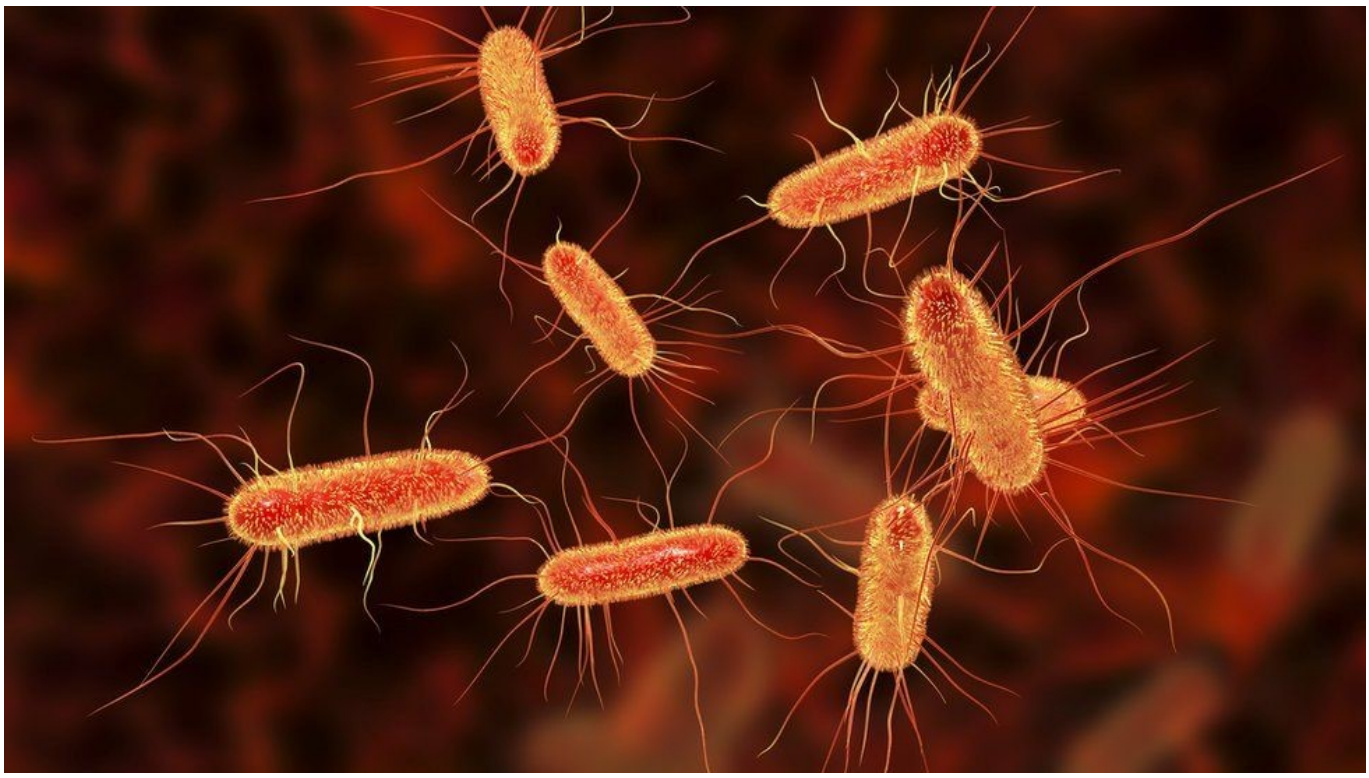
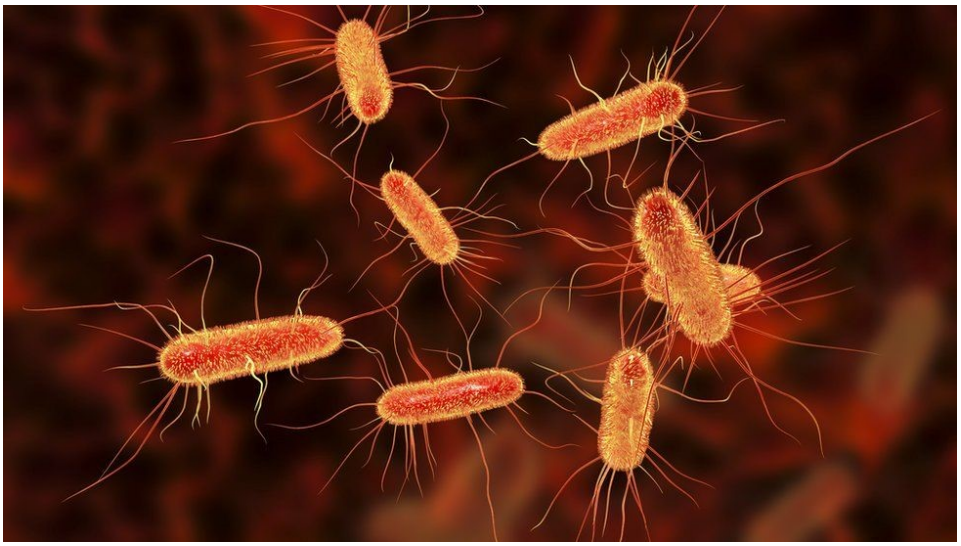
The hidden danger of endotoxins in animal production



by Technical Team, EW Nutrition

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).

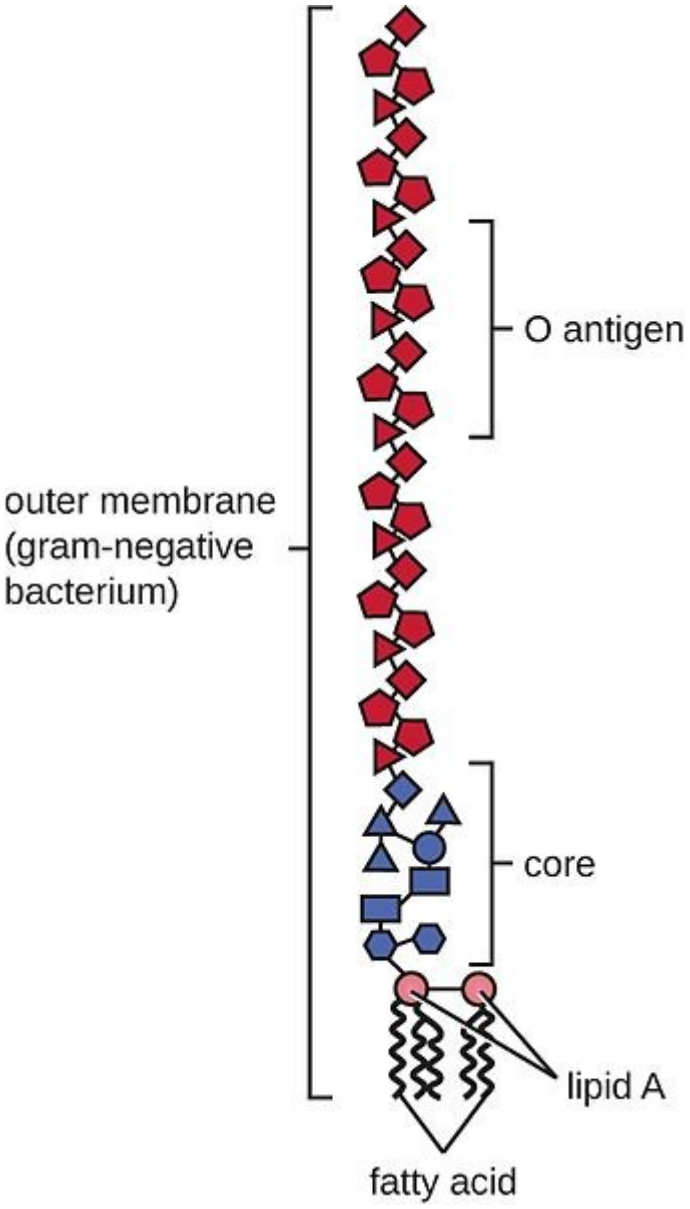


Figure 1: Structure of an LPS

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).

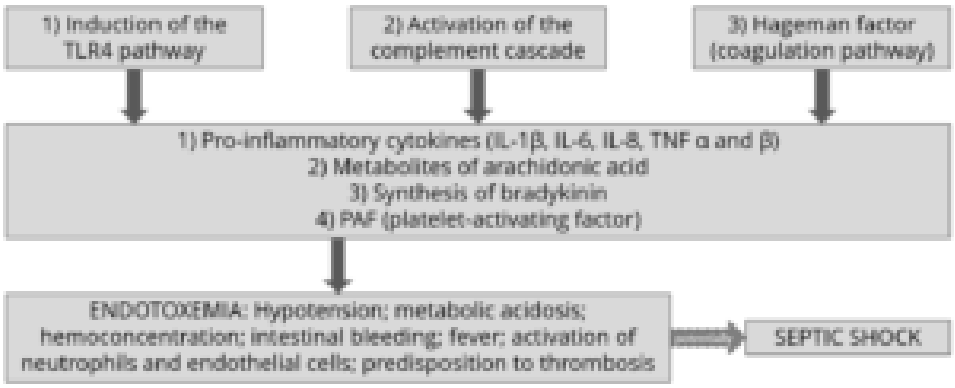


Figure 2: How LPS leads to endotoxemia – 3 modes of action

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.

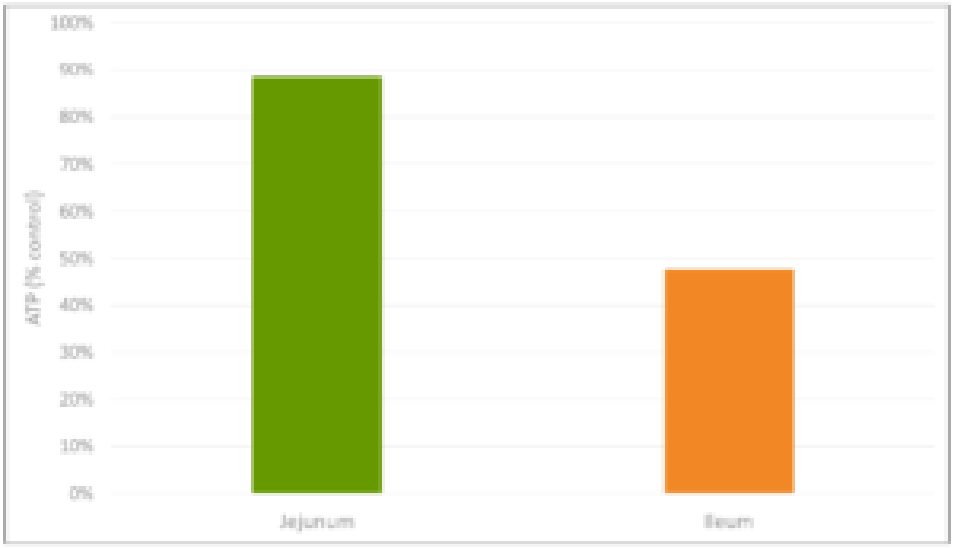


Figure 3: Reduction in ATP level in Jejunum and Ileum in broilers (adapted from Li et al., 2015)

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.

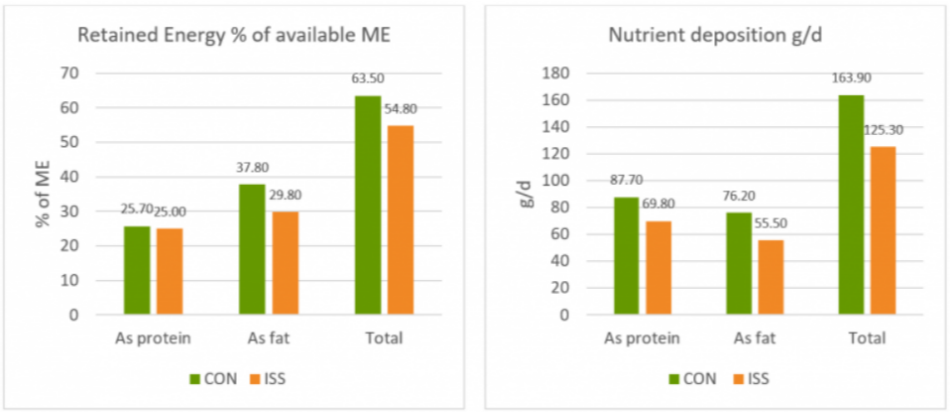


Figure 4: Retained Energy as % of ME intake and nutrient deposition of pigs in metabolic cages (adapted from Huntley, Nyachoti, and Patience, 2017)

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.

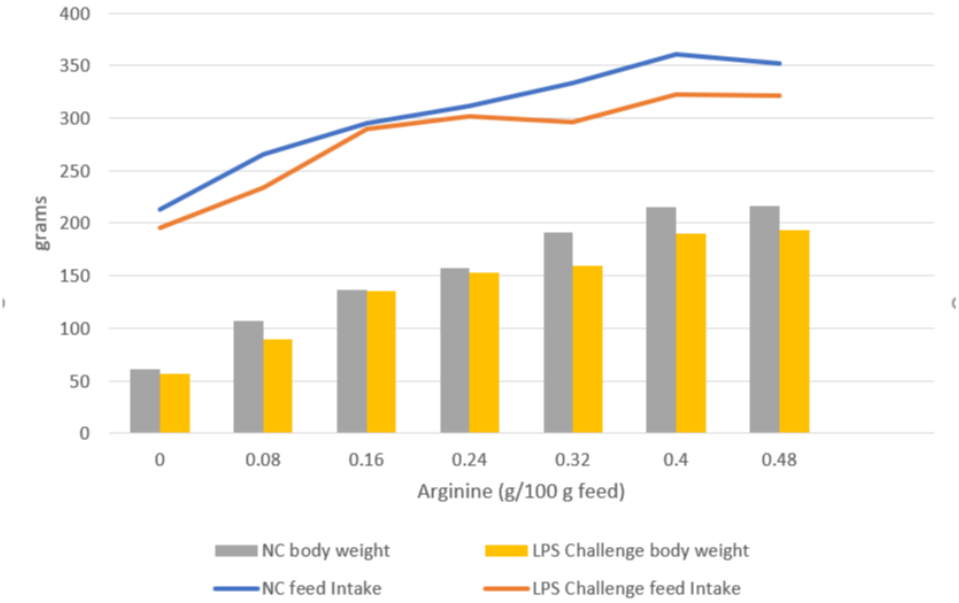


Figure 5: Effects of LPS on feed intake and body weight gain in chicks fed graded level of arginine (based on Webel, Johnson, and Baker, 1998)

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%).

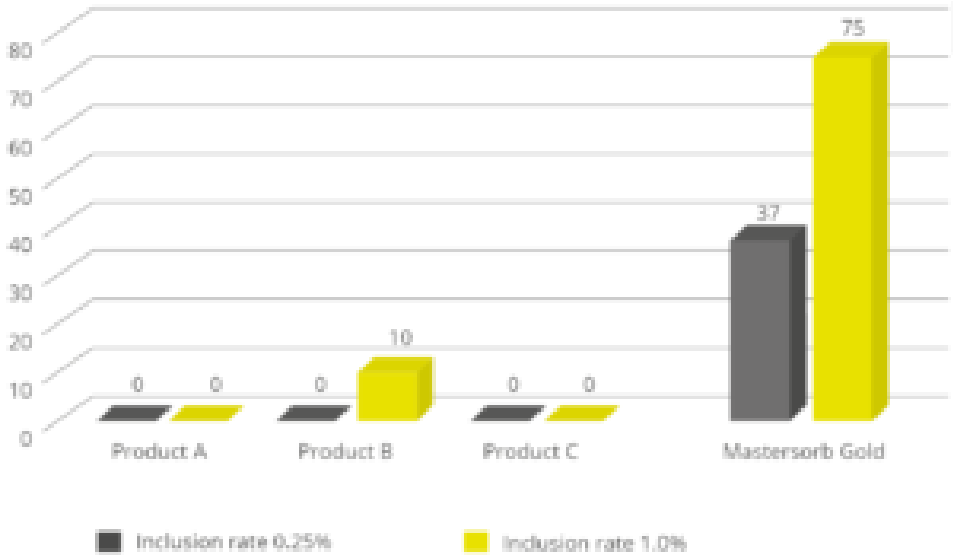


Figure 6: LPS adsorption capacity (%) – Mastersorb Gold clearly outperforms other anti-endotoxin products

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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Diarrhea? Egg powder to the rescue

Another tool to reduce the use of antibiotics is the use of immunoglobulins from eggs. Trials showed that this product is effective to support a calf's start in life and also to offer support when challenged by various forms of diarrhoea.

The main cause for calf losses during the first two weeks of life is diarrhea. In general diarrhoea is characterised by more liquid being secreted than that being resorbed. However, diarrhoea is not a disease, but actually only a symptom. Diarrhea has a protective function for the animal, because the higher liquid volume in the gut increases motility and pathogens and toxins are excreted faster. Diarrhoea can occur for several reasons. It can be caused by incorrect nutrition, but also by pathogens such as bacteria, viruses and protozoa.

Bacteria in the gut

E. coli belong to the normal gut flora of humans and animals and can be mainly found in the colon. Only a fraction of the serotypes causes diseases. The pathogenicity of *E.coli* is linked to virulence factors. Decisive virulence factors are for example the fimbria used for the attachment to the gut wall and the bacteria's ability to produce toxins.

Salmonella in general plays a secondary role in calf diarrhea, however, salmonellosis in cattle is a notifiable disease. Disease due to *Clostridia* is amongst the most expensive one in cattle farming globally. In herbivores, clostridia are part of the normal gastro-intestinal flora, only a few types can cause serious disease. In calves, *Clostridium perfringens* occurs with the different types A, C, and D. *Rotaviruses* are the most common viral pathogens causing diarrhoea in calves and lambs. They are mainly found at the age of 5 to 14 days. *Coronaviruses* normally attack calves at the age of 5 to 21 days. *Cryptosporidium parvum* is a protozoa and presumed to be the most common pathogen causing diarrhoea (prevalence up to more than 60 %) in calves.

Undigested feed and incorrect use of antibiotics

Plant raw materials (mainly soy products) are partly used in milk replacers as protein sources. These products contain carbohydrates, that cannot be digested by calves which can lead to diarrhea. The transition from milk to milk replacer can also be a reason.

An early application of tetracyclines and neomycin to young calves can lead to a change in the villi, malabsorption and therefore to slight diarrhoea. Longer therapies using high dosages of antibiotics can also lead to a bacterial superinfection of the gut. The problem is that in a disease situation, antibiotics are often used incorrectly. The use of antibiotics only makes sense when there is a bacterial diarrhea and not due to viruses, protozoa or poor feed management. To keep the use of antibiotics as low as possible, alternatives need to be considered.

Egg powder to add immunoglobulins

In order to achieve optimal results in calf rearing two approaches are possible. Firstly, the prophylaxis approach. This is the method of choice as diarrhoea can mostly be prevented. Therefore, it is necessary to supply the calf with the best possible equipment. As antibodies are one crucial but limiting factor in the colostrum of the "modern" cow, this gap needs to be minimised. A study conducted in Germany in 2015 demonstrated that more than 50% of the new-born calves had a deficiency of immunoglobulins in the blood. Only 41% of the calves showed an adequate concentration of antibodies in the blood (>10 mg IgG/ml blood serum). Immunoglobulins contained in hen eggs (IgY) can partly compensate for poor colostrum quality and serve as a care package for young animals. A trial was conducted with an egg powder product* on a dairy farm (800 cows) in Brandenburg, Germany. In total 39 new-born calves were observed until weaning (65th day of life). Before birth, the calves were already divided into control and trial group according to the lactation number of their mother cow. All calves were fed the same and received four litres of colostrum with ≥ 50 mg IgG /ml on the first day of life.

Control (n=20): no additional supplementation
Trial group (n=19): day 1 – 5: 100 g of the egg powder product per animal per day mixed into the colostrum or milk.

It was shown that the calves in the trial group showed a significantly higher (13%) weaning weight (105.74 kg compared to 93.45 kg in the control group) and 18% higher average daily gain (999 g compared to 848 g in the control group) *(Figure 1 and Figure 2).*

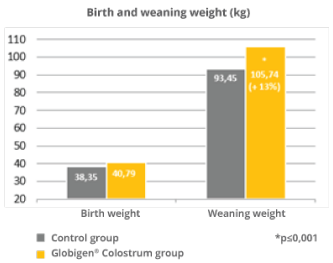

Figure 1: Effect of an egg powder product on weaning weight (kg)

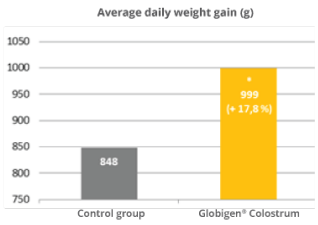

Figure 2: Effect of an egg powder product on ADG (g)

Support during acute diarrhea

When diarrhea occurs, the calf has to be treated. So the second approach is to find the best and quickest solution. It is not always necessary to use antibiotics, as they do not work against virus or protozoa. Egg antibodies can be an answer when combined with electrolytes as the following trial shows. On a dairy farm (550 cows) in Germany a feeding trial with a product based on egg powder and electrolytes** was conducted from December 2017 to May 2018. Two groups of calves were used. Before birth the animals were allocated into the two groups according to the calving plan and were examined from day one until weaning (77th day of life). All calves suffering from diarrhea (38 in total, 17 in the control and 21 in the trial group) were treated as follows:

Control (n=17): Application of electrolytes
Trial group (n=21): 50 g of the egg powder and electrolytes product twice daily, stirred into the milk replacer until diarrhea stopped.

If the diarrhea did not stop or even got worse, the animals were treated with antibiotics. It was shown that in the control group the antibiotic treatment necessary was nearly twice as long as needed in the trial group (Figure 3). This means also that nearly twice the amount of antibiotics were used. This leads to the conclusion that calves in the trial group had an improved health status compared to calves in the control group. A further result from the improved health status was an increase in performance in the trial group (Figure 4).

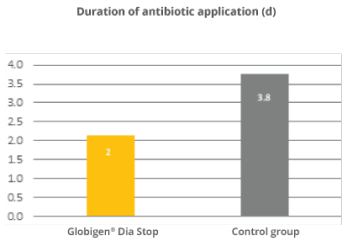

Figure 3: Duration of antibiotic application (d)

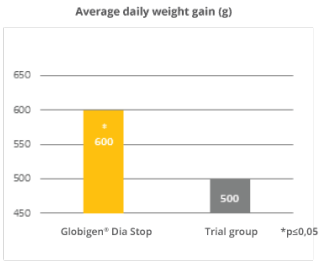

Figure 4: Effect on average daily weight gain (g)

The average daily weight gain of the trial group was 20% higher than in the control (600 vs. 500 g per day) leading to a significantly higher weaning weight (87.8 kg) than in the control (80.7 kg).

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