Masked mycotoxins – particularly dangerous for dairy cows

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Mycotoxins are secondary metabolites of fungi, commonly found as contaminants in agricultural products. In some cases, these compounds are used in medicine or industry, such as penicillin and patulin. In most cases, however, they are considered xenobiotics that are toxic to animals and humans, causing the disease collectively known as mycotoxicosis. The adverse effects of mycotoxins on human and animal health have been documented in many publications. Aflatoxins (AFs) and deoxynivalenol (DON, vomitoxin) are amongst the most critical mycotoxins affecting milk production and -quality.

Aflatoxins do not only affect cows

Aflatoxins (AFs) are highly oxygenated, heterocyclic difuranocoumarin compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They colonize crops, including many staple foods and feed ingredients. Within a group of over 20 AFs and derivatives, aflatoxin B1 (AFB1), B2, G1, and G2 are the most important naturally occurring compounds.

Among the aflatoxins, AFB1 is the most widespread and most toxic to humans and animals. Concern about mycotoxin contamination in dairy products began in the 1960s with the first reported cases of contamination by aflatoxin M1 (AFM1), a metabolite of AFB1 formed in the liver of animals and excreted in the milk.

There is ample evidence that lactating cows exhibit a significant reduction in feed efficiency and milk yield within a few days of consuming aflatoxin-contaminated feed. At the cellular level, aflatoxins cause degranulation of endoplasmic membranes, loss of ribosomes from the endoplasmic reticulum, loss of nuclear chromatin material, and altered nuclear shapes. The liver, as the organ mainly dealing with the decontamination of the organism, gets damaged, and performance drops. Immune cells are also affected, reducing immune competence and vaccination success (Arnold and Gaskill, 2023).

DON reduces cows' performance

Another mycotoxin that can also reduce milk quality and affect metabolic parameters, as well as the immune function of dairy cows, is DON. DON is produced by different fungi of the *Fusarium* genus that infect plants. DON synthesis is associated with rainy weather from crop flowering to harvest. Whitlow and co-workers (1994) reported the association between DON and poor performance in dairy herds and showed decreased milk production in dairy cows fed 2.5 mg DON/kg. However, in cows fed 6 to 12 mg DON/kg dry matter for 10 weeks, no DON or its metabolite DOM-1 residues were detected in milk.

Masked mycotoxins hide themselves during analysis

Plants suffering from fungal infestations and thus confronted with mycotoxins convert the harmful forms of mycotoxins into less harmful or harmless ones for themselves by conjugation to sulfates, organic acids, or sugars. Conjugated mycotoxins cannot always be detected by standard analytical methods. However, in animals, these forms can be released and transformed into parent compounds by enzymes and microorganisms in the gastrointestinal tract. Thus, the feed may show a concentration of mycotoxins that is still below the limit value, but in the animal, this concentration is suddenly much higher. In dairy cows, the release of free mycotoxins from conjugates during digestion may play an important role in understanding the silent effects of mycotoxins.

Fusarium toxins, in particular, frequently occur in this "masked form". They represent a serious health risk for animals and humans.

Aflatoxins first show up in the milk

Masked aflatoxins may also play a role in total aflatoxin contamination of feed materials. Research has harvested little information on masked aflatoxins that may be present in TMR ingredients. So far, metabolites such as Aflatoxin M2 have been identified (Righetti, 2021), which may reappear later in milk as AFM1.

DON-related symptoms without DON?

Sometimes, animals show DON-related symptoms, with low levels detected in the feed or raw materials. Besides sampling errors, this enigma could be due to conjugated or masked DON, which is structurally altered DON bound to various compounds such as glucose, fatty acids, and amino acids. These compounds escape conventional feed analysis techniques because of their modified chemical properties but can be released as their toxic precursors after acid hydrolysis.

Masked DON was first described in 1984 by Young and co-workers, who found that the DON content of yeast-fermented foods was higher than that of the contaminated wheat flour used in their production. The most plausible reason for this apparent increase was that the toxin from the wheat had been converted to a compound other than DON, which could be converted back to DON under certain conditions. Since this report, there has been much interest in conjugated or masked DON.

Silage: masked DON is a challenge for dairy producers

Silage is an essential feed for dairy cows, supporting milk production. Most silage is made from corn and other grains. The whole green plant is used, which can be infected by fungi. Since infection of corn with Fusarium spp. and subsequent DON contamination is usually a major problem in the field worldwide, a relatively high occurrence of this toxin in silage must be expected. The ensiling process may reduce the

amount of Fusarium fungi, but the DON formed before ensiling is very stable.

Silage samples show DON levels of concern

It is reasonable to assume that the DON biosynthesized by the fungi was metabolized by the plants to a new compound and thus masked DON. Under ensiling conditions, masked DON can be hydrolyzed, producing free DON again. Therefore, the level of free DON in the silage may not reflect the concentration measured in the plants before ensiling.

A study analyzed 50 silage samples from different farms in Ontario, Canada. Free DON was found in all samples, with levels ranging from 0.38 to 1.72 µg/g silage (unpublished data). Eighty-six percent of the samples contained DON at concentrations higher than 0.5 µg/g. Together with masked DON, it poses a potential threat to dairy cattle.

Specific hydrolysis conditions allow detection

However, in the natural ensiling process, the conditions for hydrolysis of masked DON are not optimal. The conditions that allow improved analysis of masked DON were recently described. This method detected masked DON in 32 of 50 silage samples (64%) along with free DON, increasing DON concentration by 23% in some cases (unpublished data).

Mycotoxins impact humans and animals

Aflatoxins, as well as DON, have adverse effects. In the case of DON, the impact on the animal is significant; in the case of aflatoxin, the possible long-term effects on humans are of higher relevance.

DON has more adverse effects on the animal and its performance

Unlike AFs, DON may be found in milk at low or trace concentrations. It is more associated with negative effects in the animal, altered rumen fermentation, and reduced flow of usable protein into the duodenum. For example, milk fat content was significantly reduced when cows were fed 6 µg DON/kg. However, the presence of DON also indicates that the feed probably contains other mycotoxins, such as zearalenone (ZEA) (estrogenic mycotoxin) and fusaric acid (pharmacologically active compound). All these mycotoxins may interact to cause symptoms that are different or more severe than expected, considering their

individual effects. DON and related compounds also have immunosuppressive effects, resulting in increased somatic cell counts in milk. The U.S. FDA has established an action level for DON in wheat and wheat-derived products intended for cows, which is 5µg DON/g feed and the contaminated ingredient must not exceed 40% of the ration.

Aflatoxins decrease milk quality and pose a risk to humans

Aflatoxins are poorly degraded in the rumen, with aflatoxicol being the main metabolite that can be reconverted to AFB1. Most AFs are absorbed and extensively metabolized/hydrolyzed by enzymes found mainly in the liver. This results in the formation of AFM1, a part of which is conjugated to glucuronic acid and subsequently excreted in the bile. The other part enters the systemic circulation. It is either excreted in urine or milk. AFM1 appears within 12-48 hours after ingestion in cow's milk. The excreted amount of AFM1 in milk from dairy cows usually ranges from 0.17% to 3% of the ingested AFB1. However, this carryover rate may vary from day to day and from one milking to the next in individual animals, as it is influenced by various factors, such as feeding regime, health status, individual biotransformation capacity, and, of course, by actual milk production. Carryover rates of up to 6.2% have been reported in high-yielding dairy cows producing up to 40 liters of milk per day.

In various experiments, AFM1 showed both carcinogenic and immunosuppressive effects. Accordingly, the International Agency for Research on Cancer (IARC) classified AFM1 as being in Group 2B and, thus, possibly carcinogenic in humans. The action level of 0.50 ppb and 0.05 ppb for AFM1 in milk is strictly adhered to by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), respectively.

Trials show the high adsorption capacity of Solis Max

A trial was conducted at an independent laboratory located in Spain. The evaluation of the performance of Solis Max was executed with the following inclusion levels:

- 0.10% equivalent to 1.0 kg of Solis Max per ton of feed
- 0.20% equivalent to 2.0 kg of Solis Max per ton of feed

A phosphate buffer solution at pH 7 was prepared for the trial to simulate rumen conditions. Each mycotoxin was tested separately, preparing solutions with known contamination (final concentration described in the table below). The contaminated solutions were divided into 3 parts: A positive control, 0.10% Solis Max and 0.20% Solis Max. All samples were incubated at 41°C for 1 hour, centrifuged, and the supernatant was analyzed for the mycotoxin added to determine the binding efficacy. All analyses were carried out by high-performance liquid chromatography (HPLC) with standard detectors.

Mycotoxin	Contamination Level (ppb)
Aflatoxin B1	800
DON	800
Fumonisin B1	2000
ZEA	1200

Results:
The higher concentration of Solis max showed a higher adsorption rate for most mycotoxins. The high dose of Solis Max adsorbed 99% of the AFB1 contamination. In the case of DON, more than 70% was bound. For fumonisin B1 and zearalenone, Solis max showed excellent binding rates of 87.7% and 78.9%, respectively (Figure 1).

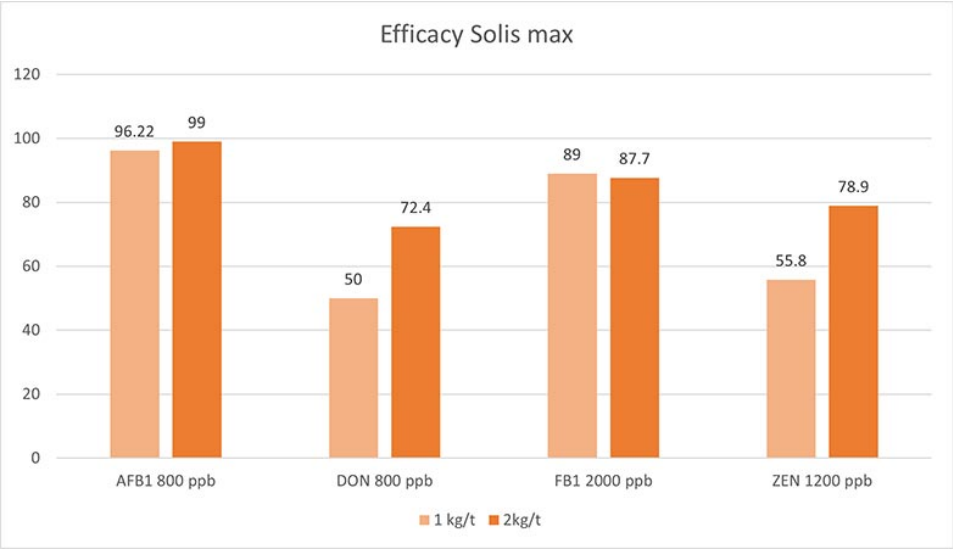

Figure 1: Solis Max showed a high binding capacity for the most relevant mycotoxins

Another trial was conducted at an independent laboratory serving the food and feed industry and located in Valladolid, Spain.

All tests were carried out as duplicates and using a standard liquid chromatography/mass spectrometry (LC/MS/MS) quantification. Interpretation and data analysis were carried out with the corresponding software. The used pH was 3.0, toxin concentrations and anti-mycotoxin agent application rates were set as follows (Table 1):

Mycotoxin	Challenge level	Challenge (ppb)	Solis Plus 2.0 inclusion	Assay time
Aflatoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.
Fumonisin	Low	500	0.2%	30 min.
	High	5000	0.2%	30 min.
Ochratoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.

Table 1: Trial set-up testing the binding capacity of Solis Plus 2.0 for several mycotoxins in different contamination levels

Results:

Under acidic conditions (pH3), Solis Plus 2.0 effectively adsorbs the three tested mycotoxins at low and high levels. 100% binding of aflatoxin was achieved at a level of 150ppb and 98% at 1500ppb.In the case of fumonisin, 87% adsorption could be reached at 500ppb and 86 for a challenge with 5000ppb. 43% ochratoxin was adsorbed at the contamination level of 150ppb and 52% at 1500ppb.

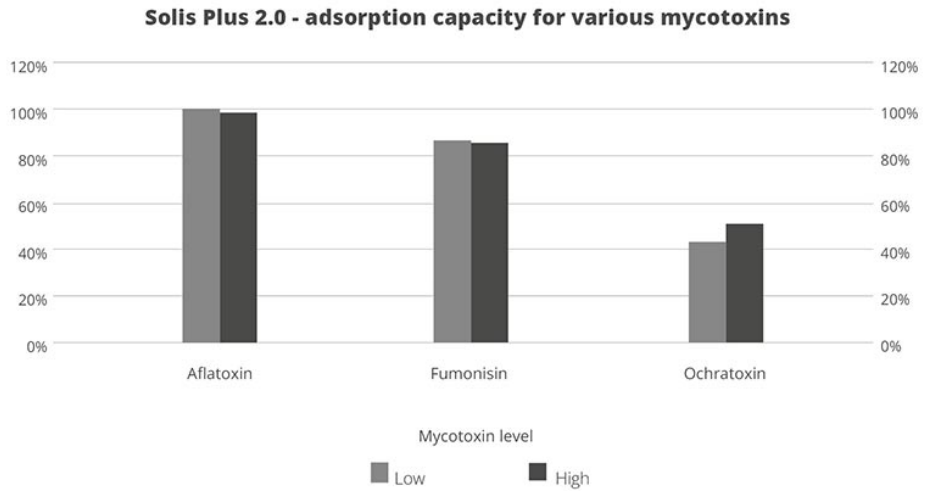

Figure 2: The adsorption capacity of Solis Plus 2.0 for three different mycotoxins at two challenge levels

Mycotoxins – Effective risk management is of paramount importance

Although the rumen microflora may be responsible for conferring some mycotoxin resistance to ruminants compared to monogastric animals, there are still effects of mycotoxins on rumen fermentation and milk quality. In addition, masked mycotoxins in feed present an additional challenge for dairy farms because they are not readily detectable by standard analyses.

Feeding dairy cows with feed contaminated with mycotoxins can lead to a reduction in milk production. Milk quality may also deteriorate due to an adverse change in milk composition and mycotoxin residues, threatening the innocuousness of dairy products. Dairy farmers should therefore have feed tested regularly, consider masked mycotoxins, and take action. EW Nutrition's MasterRisk tool provides a risk evaluation and corresponding recommendations for the use of products that mitigate the effects of mycotoxin contamination and, in the end, guarantee the safety of all of us.

Coughing calves? How to save costs and prevent respiratory disease

*By **Judith Schmidt**, Product Manager On Farm Solutions*

There will always be germs in barns. Yet, calves are particularly susceptible to lung viruses and bacteria that attack the respiratory systems. What can we do to prevent calf flu?

Coughing in calves is one of the most obvious signs of illness. It should be taken seriously – calves are important for the profitability of farms. Calf flu not only leads to treatment costs but also has long-term consequences, such as weak daily gains, delayed lactation, lower milk yield, reduced fertility, and increased susceptibility to other diseases.

Respiratory disease in calves: recognize the symptoms and protect their lung health

Calves are much more sensitive to respiratory diseases than many other animals. Why? One major cause is that calves are born with immature lungs. The lungs are only fully developed at about one year of age. In addition, calves generally have small lungs relative to their body size. Furthermore, the immunological gaps around the second month of life are decisive. During this phase, the number of maternal antibodies in the calf´s blood decreases, while the calf´s own immune system is still slowly building up.

Symptoms of calf flu

1) Cough

A very easy-to-recognize sign of a developing calf flu is coughing. Coughing can also be caused by changes in weather, stress, or an unsuitable barn climate, but coughing should always be monitored, and animals should be checked for other symptoms.

2) Respiratory distress

Sick calves breathe heavily and show an increased respiratory rate. Even at rest, this can be more than forty breaths per minute, ranging from a slight acceleration of breathing to severe respiratory distress and breathing through the open mouth. Mouth breathing can be the first indication of lung damage.

3) Eye and nose discharge

Calf flu not only shows its symptoms in the internal respiratory tract but also in the eyes and nose through clear, watery discharge. In later stages, bacterial infections can also cause purulent discharge. The animal’s gaze is not clear and rather “sleepy.”

4) Body posture

Calf flu often manifests itself by drooping ears or an overall low head posture, as the calves are dull and weak. They are inactive and separate themselves from the group. They also lie down and standing up is delayed.

5) Reduced water and feed intake

Due to their physical condition, animals suffering from flu tend to take in only little feed and water or do not eat and/or drink at all. The logical consequence is a weakening of the animals. In case of doubt, one should actively water and feed the animals.

Economic significance of respiratory disease in calves

Influenza in cattle and calves is a herd disease and often causes serious financial losses. Losses are caused by pronounced performance decreases, developmental disorders of the animals, and treatment costs. Significantly reduced daily gains have been demonstrated for fattening animals.

Next to diarrheal diseases, calf flu causes the highest treatment and follow-up costs for calves. A study by the Chamber of Agriculture of Lower Saxony (Germany) found that farmers had to spend between 83 and 204 euros per sick calf, depending on the severity of the disease.

4 tips to save costs and tackle calf flu with less antibiotics use

1) Offer a stable climate

Warm, damp barns, as well as overcrowded and poorly ventilated ones, weaken the calf´s defense mechanisms. Temperature fluctuations of more than 10°C between day and night also favor the development of calf flu. It is important to keep the calves' environment free of dust and draughts. This can be achieved by adjusting the air exchange rate.

In addition, the humidity in barns without a heating system should be between 60 and 80 percent. Data loggers help to keep an eye on the climate in the barn. They make it possible to check how the outdoor climate and ventilation affect the climate conditions in the barn.

2) Hygiene-sensitive calving management

Attention should be paid to calving management. The long-term health of the animal is already predetermined in the calving pen. If several cows calve at the same time or if calving pens are not mucked out regularly, harmful germs will accumulate. In other words: if a calf is born into a dirty box, it will absorb many germs through its mucous membranes.

3) Avoid stress

It is crucial to minimize stress from causes such as transport, re-housing, feed changes, group formation, dehorning, and weaning. These events should be spaced out as far as possible and should never occur simultaneously.

4) Prevention through supplementary feed

In the winter months, when the weather is cold and damp and constantly changing, calf flu incidence skyrockets. Now, it is imperative to strengthen the calf´s respiratory tract from the beginning. EW Nutrition's Bronchogol Liquid is a herbal concentrate that supports respiration and stabilizes the physiological defense system in the respiratory organs.

Bronchogol liquid supports young calves in stressful situations, such as critical weather transition periods (autumn-winter; winter-spring) and housing changes, and when they suffer from calf flu. The product is based on a proprietary mixture of phytomolecules. By stimulating the cilia in the respiratory tract, the phytomolecules promote the transport of mucus and facilitate expectoration.

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4 steps to improve dairy cow fertility through feeding

*By **Judith Schmidt**, Product Manager On Farm Solutions*

The average pregnancy rate for dairy cows has declined over the past decades. But why is my cow not getting pregnant? Is it because of feeding? These are questions we ask ourselves when things do not quite work out with the offspring in the cowshed. Economic success in the cow barn is closely related to the successful reproduction of our cattle herd.

The maintenance and possible improvement of fertility are becoming increasingly important issues for farm productivity. Infertility is still one of the main reasons for culling on dairy farms. When farmers decide to cull a cow after a few unsuccessful inseminations, they often ask themselves whether this could not have been prevented. There is no “all-encompassing” solution for achieving an optimal fertility rate, which ultimately requires excellent management. Relevant factors include oestrus monitoring and insemination timing, genetic conditions, feeding, hygiene, and climate.

How can I tell if a cow is in heat?

A cow behaves differently than usual during oestrus. She is restless and walks around more. A cow in heat stands next to other cows – head to tail. Sie also quarrels with her herd mates or sniffs at the shame of the other cows. Fertility in cows decreases during late winter and spring; the resulting absence of clear signs of oestrus makes it difficult to recognize the right time for insemination. There are several possible causes which will be reviewed below.

Possible causes of fertility problems in dairy cows

Beta-carotene deficiency

A productive herd needs to receive an optimal mineral and trace element supply. Beta-carotene, in particular, is essential for herd fertility. But why?

Beta-carotene is an orange-yellow plant pigment whose name comes from “carrot” because of its appearance. It is also a precursor of vitamin A. Both as a precursor and as vitamin A itself, it is essential for the organism of humans and animals, particularly when it comes to the fertility of dairy cows. Besides its important function as provitamin A, beta-carotene also exerts an independent effect on the ovary. It influences the quality of the follicle and the corpus luteum. Beta-carotene also protects the corpus luteum. It promotes the synthesis of the pregnancy hormone progesterone and thus enables the fertilized egg to implant successfully in the uterine lining.

A beta-carotene deficiency can lead to the following problems:

- Smaller, not fully functional follicles
- Altered oestrus intervals
- Indistinct signs of oestrus
- Decreased corpus luteum quality

Scientific trials show how much a beta-carotene deficiency influences the fertility process. With a beta-carotene deficiency, the fertilization rate after the first insemination is only 40%, whereas with a normal beta-carotene supply, the fertilization rate is about 70%.

How do I know if my herd is deficient in beta-carotene?

The easiest way is to check the color of the fresh colostrum. If it is a deep yellow to an even orange, the cows are supplied with sufficient beta-carotene. If it looks more ivory, this is a sign of a deficiency. Of course, a poor herd fertilization rate can also indicate a deficiency. If you suspect a beta-carotene deficiency, it is best to test some blood samples from your animal or use a testing device such as a carotene photometer. With such a test kit, you can determine not only the levels in the blood but also in the colostrum and the milk.

Feeding deficiencies

Feeding plays a major role in fertility issues. Too low input rates often have a negative effect on the health of cows. Feed quality and herd management have an impact on how long the cow loses weight after calving and at what point she gains weight again. One must always keep in mind the cows' feeding, energy balance, and nutrient supply because cows with a negative energy balance often do not show oestrus. It is also important that the silage is of high quality – poor silage inhibits fertility.

Follicle quality

The quality of the follicle is crucial for good fertility. The quality is influenced by the energy supply during the dry period and lactation during the first days. Since the follicles are already formed in the last days of gestation, a lack of energy during this period means that the maturation of the follicles – even with a better supply later on – can no longer proceed optimally and is ultimately inferior. This inevitably leads to a reduction of oestrus symptoms and minimizes the chances of successful insemination.

Prevention is key: 4 steps to improve fertility through feeding

1) Avoid stress in the feeding environment

Well-being and a high feed intake are the basis for high milk and fattening yields as well as healthy and fertile animals. Dry cows and transit cows particularly should only experience low stress. This means no overcrowding and generous feeding space, i.e., each animal should have its own feeding space. Feeding areas that are too narrow prevent the animals from eating, rank fights occur, and feed intake decreases.

Freshly lactating cows should be separated from the group. If the cows are in calving pens or calving stables, they should always have visual contact with the herd.

2) Optimize feed quality and rations

Feed quality and feeding management determine how long the cow loses weight after calving (negative energy balance) and at what point the cow gains weight again (positive energy balance). Optimal fertility performance can only happen when a positive energy balance is achieved.

The cow's fertility performance is primarily determined by nutrient supply and feeding. At the beginning of the lactation, high-quality basic feed with a high energy concentration should be fed, as feed intake is slow to get going after calving. Nevertheless, this ration should have sufficient structure. The amounts of concentrate should be divided into several individual portions and carefully increased. For high feed intakes, fresh water should be constantly available to the animals.

3) Treat diseases early to enable feeding

Diseases that lead to a reduced appetite should be treated as early as possible. In particular, attention should be paid to healthy hooves because a cow that has pain or difficulty getting up and walking is much less likely to go to the feed table.

4) Supplement vitamins, minerals, and trace elements

The needs-based supply of vitamins, minerals, and trace elements in every performance phase is a decisive success factor for good herd fertility. A sufficient supply of trace elements, especially selenium, manganese, zinc, as well as vitamin A and beta-carotene, are important for the formation of fertility hormones and optimal insemination success. At the same time, they ensure a high colostrum quality.

EW Nutrition's Fertilgol Bolus is a long-term bolus to support fertility. The high content of beta-carotene has a positive influence on the formation of the corpus luteum, the oestrus cycle, the quality of colostrum and sperm. The release rate of the ingredients beta-carotene, selenium, vitamin A, and other trace elements takes place over at least twenty days. Fertilgol Bolus can be used for female and male breeding animals shortly before and during the breeding or insemination period.

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).

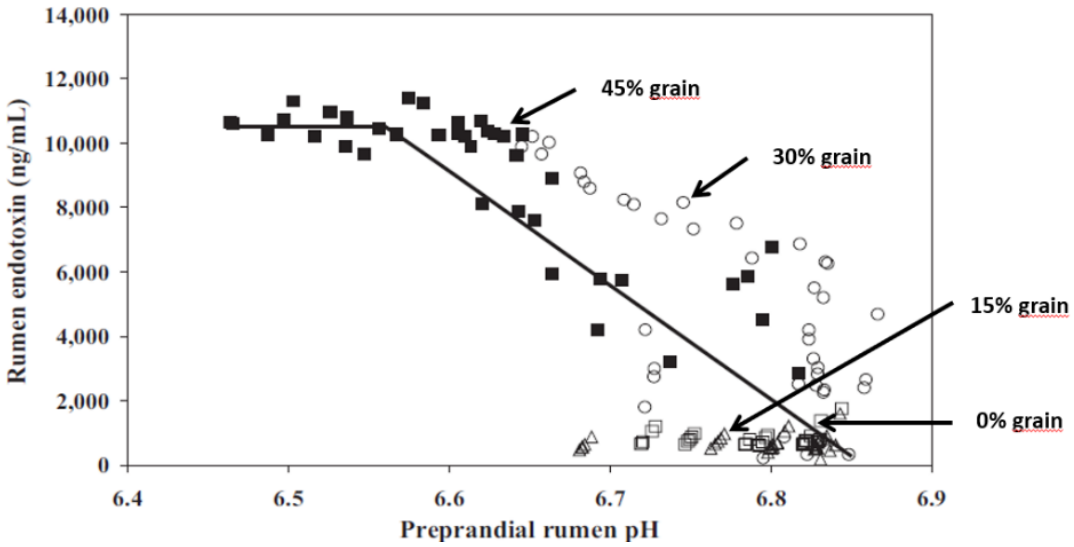

Figure 1. The increase in the level of endotoxins in the rumen is directly correlated with an increase in ration concentrates

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.

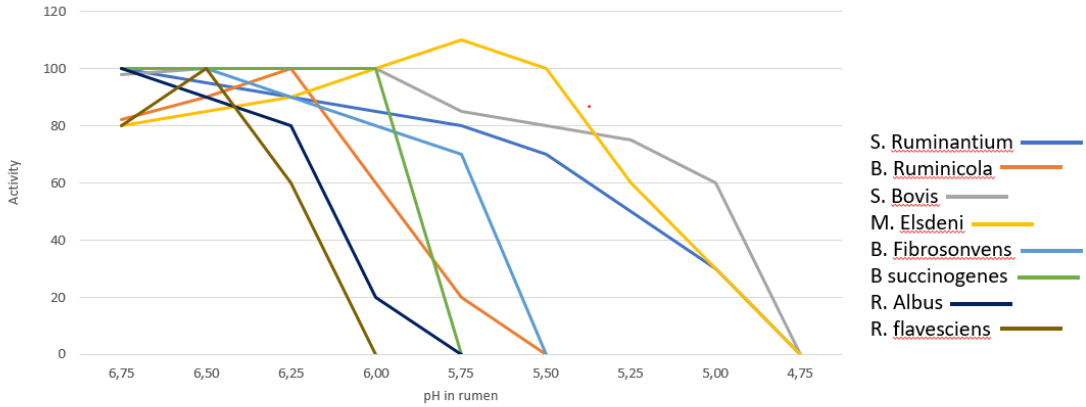

Figure 2. Activity of main bacteria in the rumen in function of pH (Daniele Cevolani Edizioni Agricole di New Business Media srl 2020)

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).

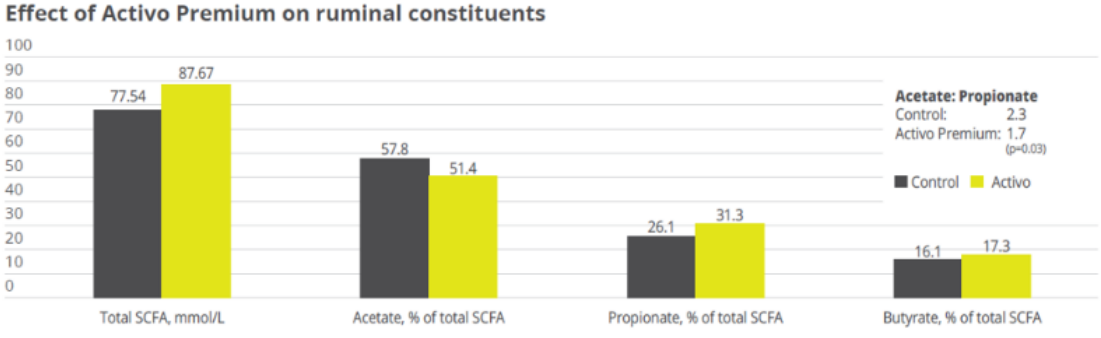

Figure 3. Effect of Activo Premium on ruminal constituents

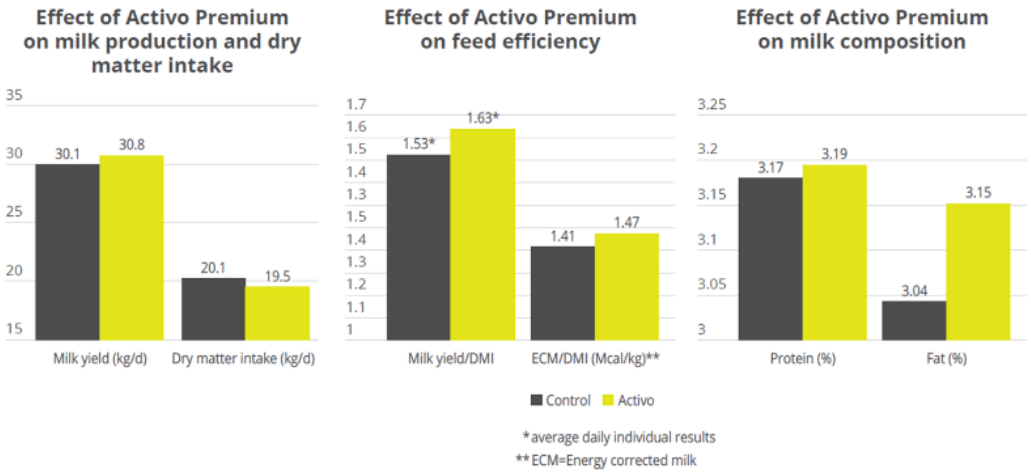

Figure 4. Effect of Activo Premium on animal performance

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.

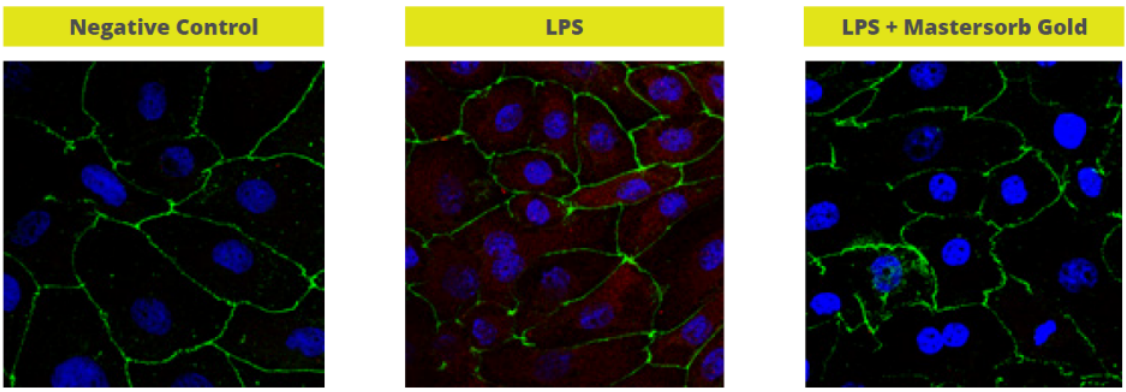

Figure 5. Immunofluorescence in PEG-J2: Challenge with LPS without (in the middle) and with Mastersorb Gold (right)

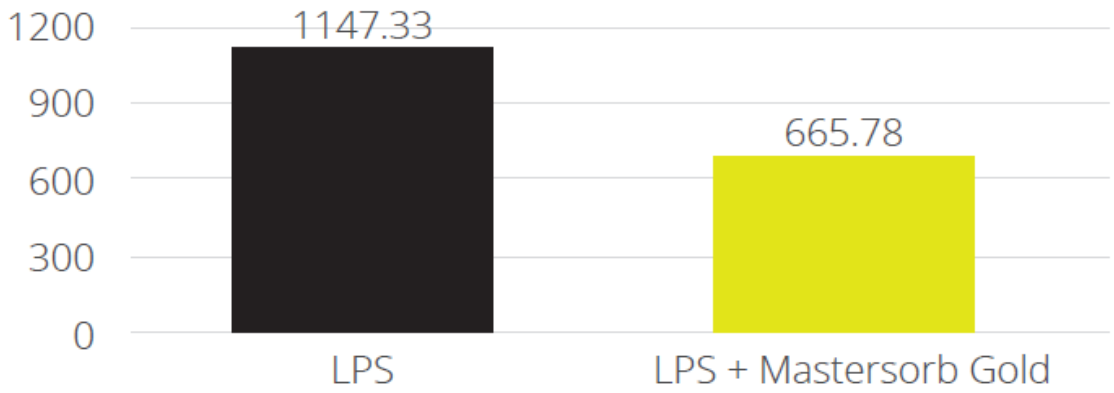

Figure 6. IL-8 AP secretion after incubation with LPS 0111:B4 for 24h without and with Mastersorb Gold

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.

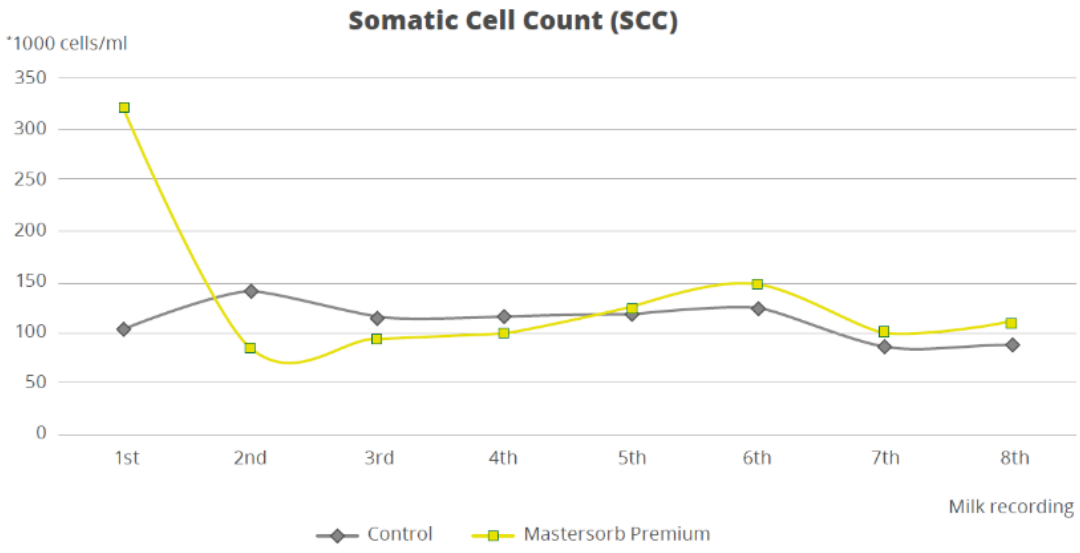

Figure 7. Effect of Mastersorb Premium on somatic cell count

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.