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Science & Solutions

What's Wrong with My Herd?
Milk Fever

Unlocking Lameness

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5 Silage Production Tips

Photo: iStockphoto.com_ekinyalgin

Stop Pathogens, Speed Up Calf Growth

Editorial

The Science Supporting Your Business

Welcome to the latest issue of **Science & Solutions**. At BIOMIN, we help you to get the most value out of feed through maximizing gut performance and protecting your animals from mycotoxins.

In this issue, Nicole Reisinger describes how BIOMIN developed a laminitis study model that provides new findings on the causes of laminitis. We provide some practical guidance on avoiding this major animal health challenge.

BIOMIN solutions are species and situation specific, so when it comes to ruminants, a large part of our focus is helping you to get silage right.

Silage is the most cost effective feed when the quality is good. In this issue, Zanetta Chodorovska covers some major points of achieving high quality silage including the crucial part of getting the microbiology right. Silage can introduce an extra dimension of mycotoxin concern as mold fungi can invade the silage. The aerobic stability provided by Biomin® BioStabil inhibits the growth of spoilage microorganisms, thus preserving the nutritive value of the silage and encouraging feed safety.

Turning to calves, we explore the success that one Danish dairy farm and local veterinarian have had supporting young animals with an enhanced acidifier added to the milk replacer.

Trials have shown that this strategy can be particularly effective in the first three weeks of the calf's life and in periods of stress, such as when calves are moved, changes in the weather, or other factors.

Finally, milk fever is not a new problem, but high-producing cows can suffer the most. Bryan Miller outlines ways to help cows cope with the sudden calcium demand following calving.

All our innovative solutions for agriculture are developed with our wide range of scientific knowledge and expertise. And this is the reason we call this magazine **Science & Solutions**.

Enjoy reading.

Vesna JENKINS
Product Manager Microbials

Contents

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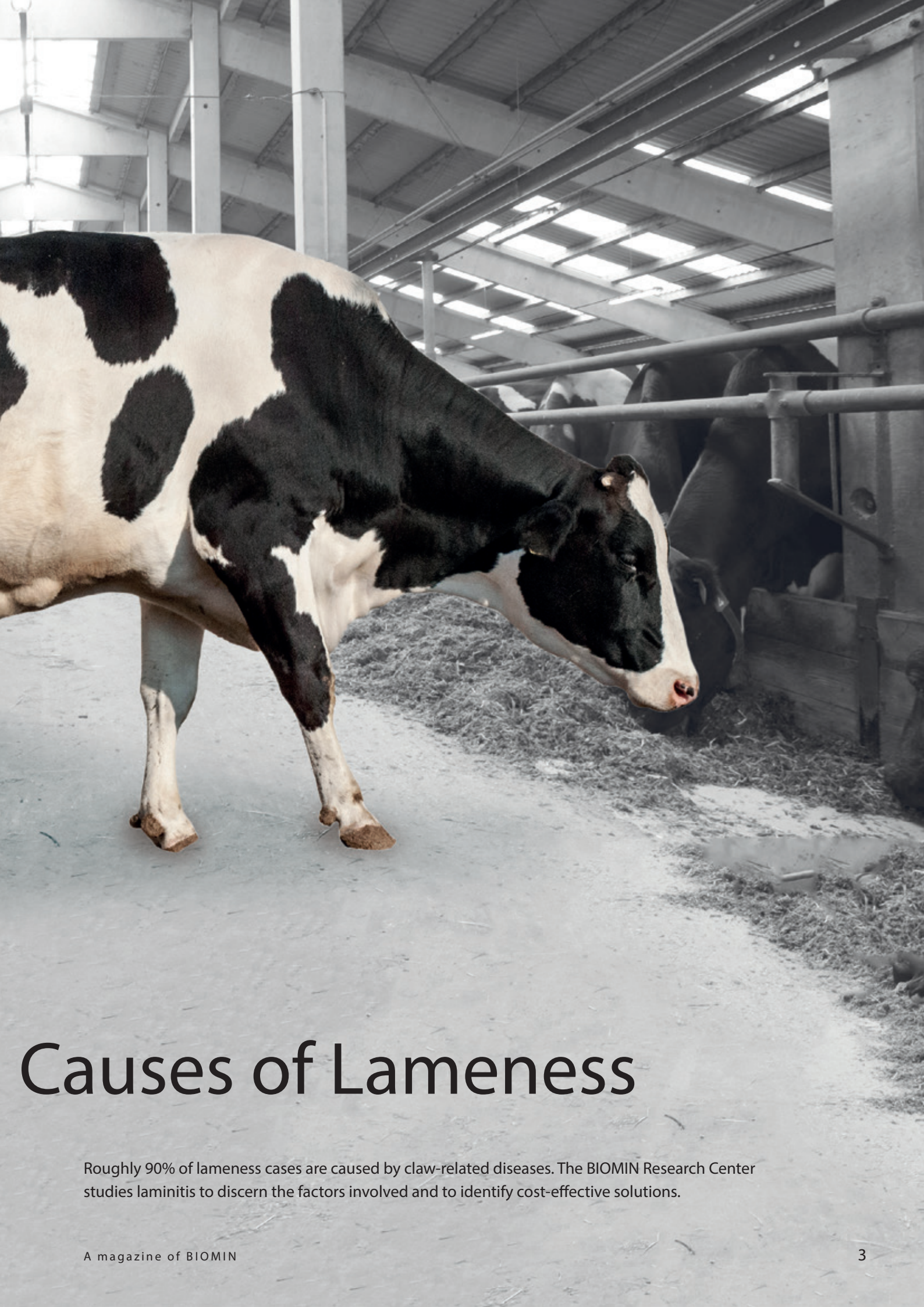
Understanding the Root

By **Nicole Reisinger**, Scientist



Causes of Lameness

Roughly 90% of lameness cases are caused by claw-related diseases. The BIOMIN Research Center studies laminitis to discern the factors involved and to identify cost-effective solutions.

Endotoxins and fumonisins have the capability

After mastitis and fertility problems, lameness is the third most important source of economic losses in dairy production. Laminitis –a disease characterized by inflammation of the lamella tissue of the claw– leads to pain for animals, greater susceptibility to other diseases, higher treatment costs, lower performance and lameness.

However, the pathology of laminitis is still not fully understood. As it is a multifactorial disease, several substances and toxins such as endotoxins are discussed as possible trigger factors. Endotoxins, or lipopolysaccharides, are cell wall components of Gram-negative bacteria that are released when bacteria multiply, lyse and die.

During bacterial imbalance in the rumen, endotoxin concentration can rapidly increase. Once endotoxins have reached the blood flow through an impaired rumen barrier, they can reach the hoof tissue and have a negative impact on tissue integrity through different mechanisms, e.g. inflammation, in which specific cells activate cytokines (e.g. TNF-alpha, IL-6) and enzymes (e.g. matrix metalloproteinases) that weaken or destroy the tissue.

In severe cases, the connective tissue of the pedal bone completely separates from the lamellar tissue—

Figure 2. Explants of about 5x5 mm contain all three important layers of the hoof/claw: connective tissue to the pedal bone (1), lamellae tissue (2) and the inner hoof/claw wall (3).

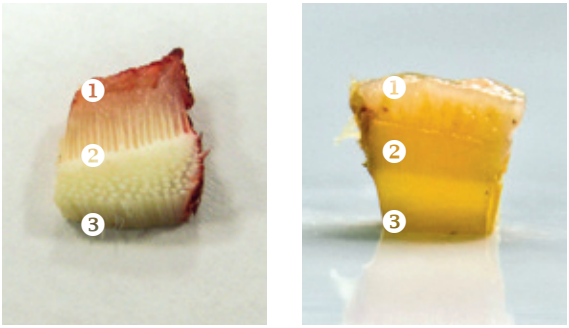


Figure 1. Overview of the dissection process of the equine hoof or bovine claw.

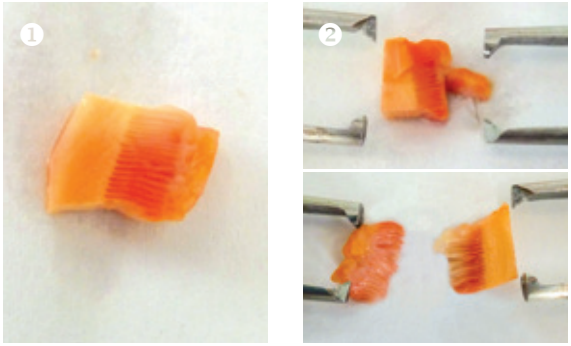
Equine hoof

Bovine claw

to aggravate the severity of laminitis.

Figure 3. Explants are cultured in cell culture plates (24 wells) with culture medium and potential trigger factors.

Figure 4. Manual separation test of explants with forceps: (1) intact explant, (2) separated explants.



causing the rotation and sinking of the pedal bone. This irreversible process causes considerable pain.

Benefits of the *ex vivo/in vitro* laminitis model

Animal experiments are associated with pain and stress for the animal. In addition, they are very time and cost extensive. An *ex vivo/in vitro* model offers an alternative way to investigate the role of different trigger factors during laminitis without the need for animal trials, and at lower costs.

From a research perspective, it allows scientists to evaluate different toxins and concentrations in one trial and to evaluate the interaction of different toxins and other trigger factors.

Furthermore, this model mimics the *in vivo* situation quite well, as all affected tissue layers are involved. The practical application aspect is also important, as it allows for the evaluation of nutrition strategies to prevent laminitis.

How the *ex vivo/in vitro* laminitis model works

Equine hooves and bovine claws are obtained from a local abattoir (horse hooves commonly serve as a model for ruminants in scientific research). The tissue is put on ice and quickly transported to the lab. Next, hooves or claws are carefully washed with a disinfectant. Initial steps of the dissection process (*Figure 1*) are performed with a band saw. Then, surgical instruments are used to prepare explants containing three layers: the inner hoof/claw wall, epidermal lamellae and connective tissue (*Figure 2*).

Finally, prepared explants are cultivated in 24 well plates (1 explant/well) with 1 mL cultivation medium at 37 °C and 5% CO_2 (*Figure 3*). During incubation potential trigger factors, e.g. toxins, can be added to each explant. Explants cultivated in medium only served as negative control.

Two different methods can be applied to evaluate if tested trigger factors have an influence on the tissue:

1. **Evaluation, if explants separate**
   Lamellar separation is tested by fixing the hoof/claw wall and the connective tissue into forceps. Explants are scored as separated, if lamellar is separated from the connective tissue or lamellar are completely destroyed; and scored intact, if not (*Figure 4*).

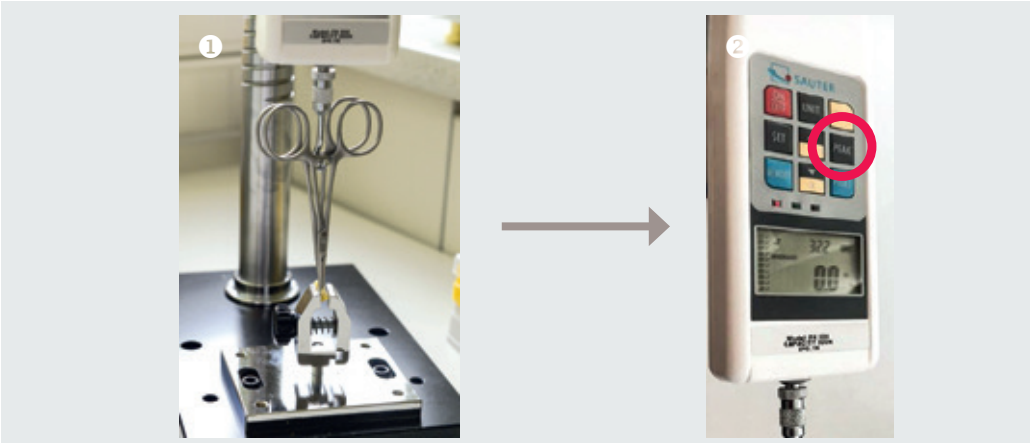
2. **Evaluation of force, which is needed to separate explants**
   The explants are fixed to a calibrated force transducer,

Table 1. Recent BIOMIN Research Center findings on laminitis.

Species	Tested toxins	Effects	Reference
Horse	Endotoxins	Significantly increased number of separated explants after 24 and 48 hours	Reisinger *et al*. 2014
Horse	Endotoxins	Significantly decreased separation force after 24 hours	Reisinger *et al*. 2015
Cow	Endotoxins	Significantly decreased separation force after 24 hours	Reisinger *et al*. 2017
Horse	Mycotoxin Fumonisin	Significantly decreased separation force after 24 hours Increase of fumonisin biomarkers (Sphinganine to sphingosine ratio)	Reisinger *et al*. 2016

Source: BIOMIN

Figure 5. Evaluation of separation force of explants. (1) explants are fixed to a force transducer, (2) maximal force is recorded, which is needed to separate explants.



and the force required for explant separation is measured (*Figure 5*).

Recent results

Recent scientific papers have shown that endotoxins and fumonisins have the capability to aggravate the severity of laminitis (*Table 1*).

Prevention tips

Our understanding of the causes of laminitis continue to advance. Here are several steps you can take to reduce the risk of laminitis in your herds:

- Appropriate feeding management: avoid excessive amounts of carbohydrates
- Proper and sufficient bedding material
- Good hygiene management
- Regular hoof/claw trimming
- Mineral supplementation
- Proper mycotoxin risk management
- Endotoxin prevention and counteracting strategies e.g. binding and bioprotection

5 Challenges to Successful Silage Production and How to Overcome Them

By **Zanetta Chodorowska**, Ruminant Technical Manager

Producing superior quality silage involves precise management of the forage from harvest right through to feed out. Here are 5 key challenges facing silage producers, and their solutions.

Higher pH levels create an environment that promotes the growth of spoilage bacteria and molds.

The optimum ensiling process should deliver a quick drop in the pH without a significant increase in temperature.

Illustrations: iStockphoto_arcady_31 / Alex Belomlinsky

Silaging, also known as ensiling, is used by farmers all over the world to preserve forage. It is a common technique based on anaerobic (without oxygen) fermentation. Harvested forage is wilted—especially if it is low in dry matter content and chopped before being compacted and stored in silos, bunkers or other types of enclosed environments. These are covered and made airtight to prevent oxygen getting in, providing the ideal conditions for anaerobic fermentation.

At the start of the fermentation process, the forage is still a living material with plant cell respiration occurring. Also, the crop is covered with microbes. Most microbes are Gram-negative, aerobic (oxygen needing) species with fewer anaerobic species present. When residual plant respiration and the metabolism of microorganisms depletes the remaining oxygen from the forage, anaerobic conditions are created and the fermentation process can start.

For effective fermentation, the aerobic microbes need to be replaced with anaerobic, Gram-positive, lactic acid producing bacteria. This can be achieved by adding bacteria capable of dominating the entire process with minimal nutrient losses. Such bacteria are present in Biomin® Biostabil products. Biomin® Biostabil Maize should be used on corn based forage crops, and Biomin® Biostabil Plus should be used for grass, alfalfa and clover based crops.

With feed costs accounting for 50% to 70% of dairy farm inputs, and forage accounting for 40% to 60% of the ration, it is vital to ensure the quality of the forage produced on farm. There are many sources of spoilage which we will explore further here.

1. Temperature

The optimum ensiling process should deliver a quick drop in the pH without a significant increase in temperature. A slight increase up to 37°C is accepted at the beginning of the process when the plant is still respiring, but prolonged and elevated temperatures cause significant loss of nutrients from the ensiled material. However, in warm climates, an elevated temperature may persist in the silage for several months.

An increase in the temperature of the silage results in 1) a loss of energy through CO_2 loss, 2) a decrease in nutrient availability, and 3) a decrease in the palatability of the silage material that diminishes intake by animals.

2. pH levels

Heterofermentative lactic acid bacteria (e.g. *L. kefiri*, *L. brevis*, and *L. buchneri*) produce lactic acid and acetic acid within a month of ensiling. Higher pH levels create an environment that promotes the growth of spoilage bacteria and molds, thereby

Zanetta Chodorowska
Ruminant Technical Manager

The negative impact of yeast can be reduced by having a proper dry matter content at harvest, cut length, good compaction at storage, and proper feed out methods.

Table 1. Negative effects of yeast in the aerobic and anaerobic phases of silage production.

	Aerobic conditions	Anaerobic conditions
Mode of action	Produce acetic acid and aromatic aldehydes	Ferment plant sugars to CO_2 and ethanol
Result	Change in smell which reduces intake.	Unpleasant smell reduces intake.
	Raised temperature and pH leading to spoilage	Reduction in energy and dry matter content.

Source: BIOMIN

heightening the risk of mycotoxin contamination occurring. Applied fermentation bacteria (e.g. *L. plantarum* and *L. brevis*) utilize plant sugars to produce lactic acids within the first 1-2 weeks that stabilize silage at a terminal pH. In turn, this lower pH inhibits spoilage microorganisms that are sensitive to low pH levels. Once a clump or silo is opened, the lactic acid present in the silage would be consumed by aerobic yeast—were it not for the acetic acid which acts as a growth inhibitor.

3. Yeast

The negative effect of yeast in silage is often underestimated. Yeasts thrive in both the aerobic and anaerobic phases of silage production. Weather conditions at harvest have a dramatic effect on the number of yeast on fresh forage. Those present at harvest requiring oxygen for respiration will be reduced by the anaerobic phase during storage, and will also be present during feed out at the end of the process. The yeasts are 90% sugar utilizers as well as 90% acid utilizers. The sugar-utilizing yeasts dominate at the beginning of the process during the aerobic ensiling and storage phases. The acid-utilizing yeasts dominate during the feed out phase and are responsible for the aerobic deterioration of the silage. Their activity results in an increase in temperature, reduction of fermentation acids, and elevation of silage pH (*Table 1*).

The negative impact of yeast can be reduced by having a proper dry matter content at harvest, cut length, good compaction at storage, and proper feed out methods.

The use of silage inoculants containing heterofermentative strains such as *L. kefiri* and *L. brevis* as contained in Biomin® Biostabil will produce a small amount of acetic acid during fermentation, therefore inhibiting the yeast.

An efficient and quick reduction of the pH during ensiling will prevent the growth of *Clostridia*.

Photo: iStockphoto.com_decade3d

70-90% of molds and mycotoxins are present on the plants at the time of harvest.

Field fungi (e.g. *Aspergillus* and *Fusarium* spp.) are capable of producing mycotoxins which will cause health problems when fed to animals.

4. Clostridia

In high moisture material, the biggest enemy is *Clostridia*; an anaerobic endospore-forming bacteria. During harvesting and ensiling, *Clostridia* contaminates the crops and enters the silage bunkers and pits via either 1) manure from fertilized fields or 2) the soil (e.g. from rain splashing during wilting, loose soil released by machinery).

Clostridia grows only in anaerobic conditions, fermenting sugar, protein and amino acids into butyric acid and ammonia as well as toxic amines. The products of clostridial fermentation are responsible for depressed feed intake, increased risk of ketosis, hemorrhagic bowel syndrome (HBS), and sudden death of animals. Feeding silage with elevated levels of butyric acid should be avoided, especially for cattle at sensitive stages e.g. early lactation.

An efficient and quick reduction of the pH during ensiling will prevent the growth of *Clostridia*. It has also been proven that lactic acid producing bacteria, such as *L. brevis* as found in Biomin® Biostabil, can inhibit butyric acid formation in ensiled material.

5. Molds

70-90% of molds and fungal origin mycotoxins are present on the plants at the time of harvest and enter the clamps and silos with harvested material. They enter growing plants through the roots during the seedling stage, travel down through either the silk channels during pollination or through plant wounds from environmental or insect injury. Field fungi (e.g. *Aspergillus* and *Fusarium* spp.) are capable of producing mycotoxins, including aflatoxin, deoxynivalenol (vomitoxin), fumonisin, zearalenone and T-2 toxin, which will cause health problems when fed to animals. None of the silage additives or inoculants available on the market today are able to degrade field origin mycotoxins as they are resistant to low pH and anaerobic conditions.

In aerobically challenged silages, fungi that have developed during the storage phase will start to produce additional so called 'silage born' toxins when they are exposed to the air during feed out.

Penicillium spp. which are typically bluish-green in color and their toxins (e.g. PR toxin, patulin, citrinin, mycophenolic acid and roquefortine C) are of greatest concern in ensiled forages. It is recommended to screen silage in a lab for mycotoxin contamination on a regular basis, or at least each time there is a reduction in feed intake. Once mycotoxins are detected, or are highly suspected from fungi identification, the ration should be supplemented with Mycofix® Plus. For more information and technical support for your forage production, please contact your local BIOMIN representative.

A Feed Additive to Stop Pathogens, Speed Up Calf Growth

Text and photos by **Claus Solhøj**

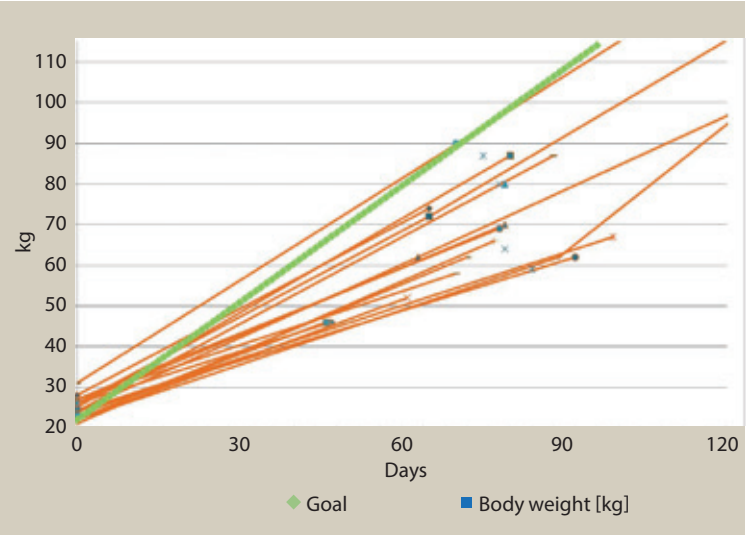
A dairy farm in Munchgaard, Denmark obtained, on average, an extra 15kg of growth in Jersey cow calves in the milk-feeding period by adding the pathogen control additive, Biotronic® PX Top3.

During the milk-feeding period, growth has increased, on average, by 15kg in Jersey and crossbred calves. This is the result of adding Biotronic® PX Top3 to the feed at Munchgaard. Anni Høegh Christensen looks after the calves at the farm, and she can give precise figures, because the calves are weighed at birth and again when they are weaned and when milk feeding ends.

This means that it is well worth taking the extra trouble to mix Biotronic® PX Top3 with the milk replacer diet adjusted for the dry solids content, and accepting the slight extra cost per day of adding 6g per calf per day

Niels Ole Sørensen and Anni Høegh Christensen, who is in charge of the calves, by the outdoor calf stall with individual boxes.

Figure 1. Weight increase control at Munchgaard during the milk feeding period after using Biotronic® – close to the target.



during the milk-feeding period. It meant better health, less work looking after the calves, increased growth, and finally, calving at an earlier age and stronger dairy cows.

The cows are needed on this farm in West Zealand, Denmark, where Niels Ole Sørensen is responsible for farming the 320 hectares of land, while his brother, Jens Christian Sørensen, is in charge of the dairy area, which is expanding from the original production of 200 cows to 500, and converting from Danish Holstein to Jersey to exploit the facilities better. They are expanding with their own supplements, so every single dairy calf is very welcome.

Biotronic® PX Top3 was introduced at Munchgaard by the veterinarian, Rikke Engelbrecht (PhD), who worked on trials with the West Jutland agricultural association Vestjysk Landboforening in 2015 and 2016, and was able to show a significant reduction in diarrhea in calves during the milk-feeding period, especially when the challenges came from *Salmonella* spp. and *E. coli*. The effects had been seen in pigs and poultry, but it was something new that the bacteriostatic effect was also seen in calves.

The reduction in calf diarrhea alone means increased growth, and this is increased further by a reduction of the pathogenic load in the milk and gastrointestinal tract.

Rikke Engelbrecht recommends using Biotronic® PX Top3 from day one in a calf's life, or when feeding with colostrum ends, as it gives the milk a flavor that can lead to refusal if its use is started later.

A feature of calf husbandry at Munchgaard in the winter months consists in giving calves in the milk-feeding phase lukewarm water with added electrolyte and glucose twice a day.

Mixing is best done separately in water at 50°C or in a little of the mixed milk replacer. It can then be added to the mixed milk replacer and fed to the calves immediately afterwards. Rikke Engelbrecht's trials show that the greatest effects are seen in the first three weeks of the calf's life, when it is most vulnerable, and in periods of stress, such as when calves are moved, changes in the weather, or other factors.

This article originally appeared in LandbrugsAvisen

Cut & Keep Checklist

What's Wrong with My Herd? Part 4: Milk Fever

Milk fever, also known as hypocalcaemia or parturient paresis, is by no means a new condition for modern dairy cattle. Calcium, in addition to being a major component of bone, is also needed metabolically to transfer the message for both skeletal and smooth muscles to contract. A shortage of calcium can result in tremors, cows found in a 'sitting' stance, eventual collapse, and potentially death.

Timing and susceptibility

Milk fever can be caused by the large need for calcium for the production of colostrum. As a result, about 80% of milk fever occurs within one day of calving.

Older cows (two or more lactations) seem to be more likely than first calf heifers to have milk fever, but cows of any age are susceptible. Additionally, Jersey cattle are more predisposed to milk fever than other breeds. Milk fever is most common in high producing dairy cows. An incidence of 5% is not unusual, but incidences over 10% certainly indicate a larger problem needing specific management changes.

The incidence of milk fever can increase due to the presence of other common metabolic disorders. Milk fever incidence is greater in over-conditioned cows. This is most likely related to clinical and subclinical ketosis which reduces feed intake postpartum and puts additional pressure on an already limited calcium supply.

Related issues

Milk fever can also predispose the cow to other metabolic disorders and infectious diseases. Cows with milk fever may have greater plasma cortisol levels which can cause immunosuppression.

Additionally, the cells' internal calcium is used as a secondary signal once outside of the cell to stimulate an immune response.

Lowered plasma calcium concentrations, as in milk fever, can result in reduced cellular calcium concentration and a blunted immune response. It is not uncommon to have increased cases of mastitis and metritis in cows that have suffered from milk fever.

Milk fever can also contribute to dystocia, retained foetal membranes, and uterine prolapse. Additionally, cows will have reduced feed intake which can lead to more cases of ketosis and displaced abomasum.

Feed recommendations

Low calcium diets (less than 20g per head per day) have been implemented by some producers successfully, though it can be difficult to formulate rations with such low levels of calcium. Proper attention to dietary anion strength is a better way to manage diets to reduce milk fever. Dietary Cation-Anion Difference (DCAD) diets balance four macrominerals: the anions chloride and sulphur; and the cations sodium and potassium. This balance can help determine blood (and urine) pH. Slightly acidic conditions are needed for the proper mobilisation of bone so that calcium can be released for colostrum and milk production.

Diets designed for dry cows in the weeks prior to calving should have a negative DCAD. The goal is to lower blood pH. Fortunately, producers can easily monitor pH via the urine. A pH of 7.0 or greater would suggest that a producer should consider balancing for cations-anions. Proper balance does require regular monitoring as producers should not let urine pH drop below 5.5. A urine pH of 6.0 to 6.5 indicates an effective DCAD diet.

Potassium content of forages can greatly affect DCAD: increased potassium content is a contributor to milk fever. Also, as temperatures rise, cows pant more, expelling more CO_2 resulting in lower pH. Products used to increase the negative balance include magnesium sulphate, calcium sulphate, ammonium sulphate, calcium chloride, ammonium chloride, and magnesium chloride. Many of these products can be unpalatable. Producers are cautioned to ensure dry matter intake is not negatively affected. Protein sources treated with hydrochloric acid provide an additional way to increase negative charges and avoid some of the palatability problems associated with anionic salts.

Further steps

In addition to managing calcium in the diet and blood pH, producers need to consider the overall herd management in regards to feed intake, energy balance and other challenges.

Maintaining feed intake through diet balance and potential intake enhancers such as yeast products and phytogenic products can have the additional benefit of reducing the effects of milk fever.

Reducing other challenges to the cow, including pathogens and mycotoxins, should help reduce the secondary effects of milk fever that can impact disease status and reduce milk production.

Dietary Cation-Anion Difference

The pH can be manipulated by controlling the concentration of sodium (Na), potassium (K), chloride (Cl), and sulphur (S) that the cows consume.

What actually is determined is based on the charges of each anion (Cl and S) and cation (Na and K) mineral. The equation below takes into account the molecular weight of the respective minerals.

DCAD Equation

Sodium (Na) x 435 + potassium (K) x 256 - chloride (Cl) x 282 + sulphur (S) x 624 = milliequivalents (mEq)/kg dietary dry matter

References are available on request

For more information, visit **www.mycotoxins.info**

DISCLAIMER: This table contains general advice on matters which most commonly affect ruminants and may be related to the presence of mycotoxins in feed. Ruminants diseases and problems include, but are not confined to the ones present in the table. BIOMIN accepts no responsibility or liability whatsoever arising from or in any way connected with the use of this table or its content. Before acting on the basis of the contents of this table, advice should be obtained directly from your veterinarian.

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