Newsletter

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> EDITORIAL

In March 2010, breaking news disturbed the peaceful life in Austria: eight people had died and numerous people had fallen ill after consuming a certain type of cheese.

In a country with excellent sanitary conditions and extremely strict food quality controls, this was inadmissible. Following investigations, the sanitary authorities found that the disease was caused by the infectious agent Listeria monocytogenes (see Picture 1).

In the newspaper clipping shown in Figure 1, two German words are marked with red boxes: Giftkäse means poisonous cheese and Listerien means Listeria. Two words that are unfortunately often seen together.
But what does all this have to do with a newsletter about silage inoculants? Silages are the end product of the anaerobic fermentation of very different substrates (crops as grass, corn, alfalfa; but also brewer's grains, beet pulp, etc.).

Silages are normally a wet feedstuff (30 – 80 % moisture) that is rich in nutrients; excellent conditions for the growth of microorganisms. Are these microorganisms beneficious? Are some of them harmful? How can we stop/ eliminate them? Can silage inoculants contribute to the prevention of diseases caused by infected silages?

I invite you to read the following newsletter to find the answers to the questions above!

Best wishes,

Competence Center Microbials

Biomin® BioStabil

The main infectious microorganisms present in the silages (*Listeria monocytogenes, Clostridia_ Clostridium botulinum, Entereobacteriae_Escherichia coli, yeasts and moulds*) are microbes that have different characteristics (classification physiology, pathogenesis, detection, epidemiology, routes of infection, infectious cycles, etc.). Good agricultural practices can help prevent infections transmitted by the ingestion of contaminated silages.
The aim of this newsletter is to analyze the possible control of harmful microorganisms present in silages using inoculants.

The contribution of silage inoculants to disease prevention*

** The main results outline in this newsletter were presented at the "14th International Conference on Production Diseases in Farm Animals" in June 2010, in Gent, Belgium.*

CHARACTERIZATION OF HARMFUL MICROORGANISMS AND THEIR RELEVANCE IN SILAGES.

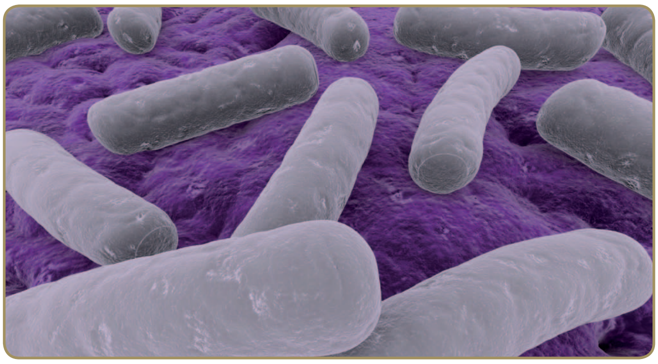

Neuer Vorwurf an Quargel-Firn
Giftkäse wissentlich ausgelief
Der Fall um mit Listerien verseuchten Käse in Österreich wird immer unheimlicher: Nicht nur, dass große Mengen des bakterienbefallenen Käses an Sozialmärkte geliefert wurde, nun deckte das
te – „Heute" berichtete
Für Gesundheitsminis
ger ist das ein Fall für di

Picture 1: *Clipping from an Austrian newspaper*(0)

Listeria monocytogenes: gram-positive bacterium that can move within eukaryotic cells (see *picture 2*). Clinical symptoms are frequently recognized by veterinarians, especially as meningoencephalitis, abortions and mastitis in ruminants. The bacterium lives in the soil and in poorly made silage and is acquired by ingestion. It is not contagious; over the course of a 30-year observation period of sheep disease in Morocco, the disease only appeared in the late 2000´s when feeding bag-ensiled corn became common. In Iceland, the disease is called silage sickness[1]. *L. monocytogenes* cannot usually survive below pH 5.6 but in poorly consolidated silage with some oxygen present it can survive at pH levels as low as 3.8. As these conditions also favor the growth of certain moulds, moldy silage generally presents a high risk of listeriosis[2].

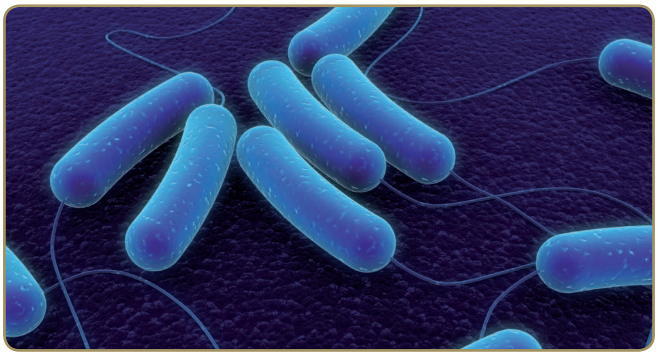
Picture 2: *Listeria monocytogenes*

Clostridia: gram-positive obligate anaerobic bacterium that can form spores (see *picture 3*). Crops for ensiling are often harvested in relatively wet conditions and have a low dry matter content (<25 %). This presents a risk of contamination with *Clostridia*, which increase the nutrient (protein) losses in silages and cause fermentation to become butyric. An important consequence is also the rejection by the animals because of the low silage palatability. *Clostridia* can be stopped by a quick and deep pH decrease (pH below 4.5[3]).



Picture 3: *Clostridia*

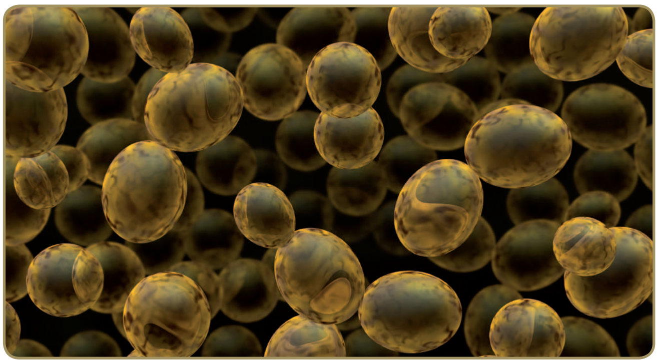
Entereobacteriae (coli forms): gram-negative, non-spore forming, facultative anaerobe (see *picture 4*). They commonly enter silage from slurry, manure and soil in the early stages of fermentation and convert the water-soluble carbohydrates into acetic acid, ethanol, CO_2 and ammonia with high energy losses. Their growth is decreased by anaerobiosis, low pH values and fermentation acids. The optimal pH value for growth is around 7; lower pH values markedly decrease the growth[4].



Picture 4: *Coli bacteria*

Yeasts: eukaryotic unicellular aerobic microorganisms (fungi) that use organic compounds as a source of energy, mostly from hexoses and disaccharides, and do not require sunlight to grow (*picture 5*). There are no known yeast species that only grow anaerobically (obligate anaerobes)[5]. Yeasts grow best in a neutral or slightly acidic pH environment.

During the feed out phase in the absence of inhibiting substances like acetic and propionic acid, yeasts can grow very rapidly and surpass 1 000 000 cfu/g silage, not only causing aerobic instability (5) but also increasing the risk of diarrhea in domestic animals. They compete with the lactic acid bacteria for sugars, which they ferment to mainly create ethanol. Ethanol has little (if any) preservative effect in the silage but causes extremely dry matter and high energy losses (48.9 and 0.2 % respectively[6]). A level of acetic acid of 1.5 – 3.0 % in the dry matter could stop the yeast growth in the silage exposed to air in the feed out phase[7]. However, higher levels diminish the silage palatability. An overview of results in the scientific literature about inhibition of yeast by acetic acid is presented in *Table 1*.



Picture 5: *Yeasts*

Table 1: *Effect of acetic acid on different yeasts*

Microorganisms	Author	Year	Statement
Saccharomyces rouxii and Toru-lopsis versatilis	Noda *et al.*	1982	An increased toxic effect in brine fermentation of soy sauce from ph 5.5 to 3.5
Candida krusei and Pichia subpelliculosa	Danner *et al.*	2003	Acetic acid has the greatest inhibitory effect on yeast growth. 20 g liter^{-1} of acetic acid in the test mixture was enough to completely inhibit the growth of the selected yeasts at pH 4.
Silage yeasts	Driehuis and van Wikselaar Oude Elferink *et al.*	1996 1999	High levels of formic or acetic acid reduce survival during storage (in silages)
Silage yeasts	Driehuis et al. Oude Elferink *et al.*	1997 1999	Lactic acid is degraded anaerobically to acetic acid and 1,2-propanediol, which in turn causes a significant reduction in yeast numbers

Table 2: The control of harmful microorganisms present in silages

Parameter	Microorganismus				
	Listeria monocytogenes	Clostridia	Entero-bacteriae	Yeasts	Moulds
Nutrients (Water soluble carbohydrates)	+++	+++	+++	+++	-
Anaerobiosis	+++	-	+++	+++	+++
pH*	+++	+++	+++	-	-
Lactic acid* (fermen-tation)	+++	+++	+++	-	-
Acetic acid* (feed out phase)	+	+	++	+++	+++

- Low inhibition, + High inhibition.

** Factors influenced by the use of silage inoculants*

Moulds: grow in multicellular filaments and derive energy from the organic matter in which they live (see *picture 6*). Mould spores can remain airborne indefinitely, live for a long time, cling to clothing or fur, and survive extremes of temperature and pressure. Many moulds also secrete mycotoxins, which, together with hydrolytic enzymes, inhibit the growth of competing microorganisms. Those toxins can negatively affect the performance of domestic animals. Milk contamination, decreased milk production, mastitis, laminitis, poor reproductive performance and several gastrointestinal disorders are well described in dairy cattle. The main mycotoxins founds in silages were ZON, DON and fumonisin[8] as well as roquefortine. The majority of fungi are strict aerobes (require oxygen to grow) [9]; only few of them are microphilic (*Mucor spp.* for instance [10]).

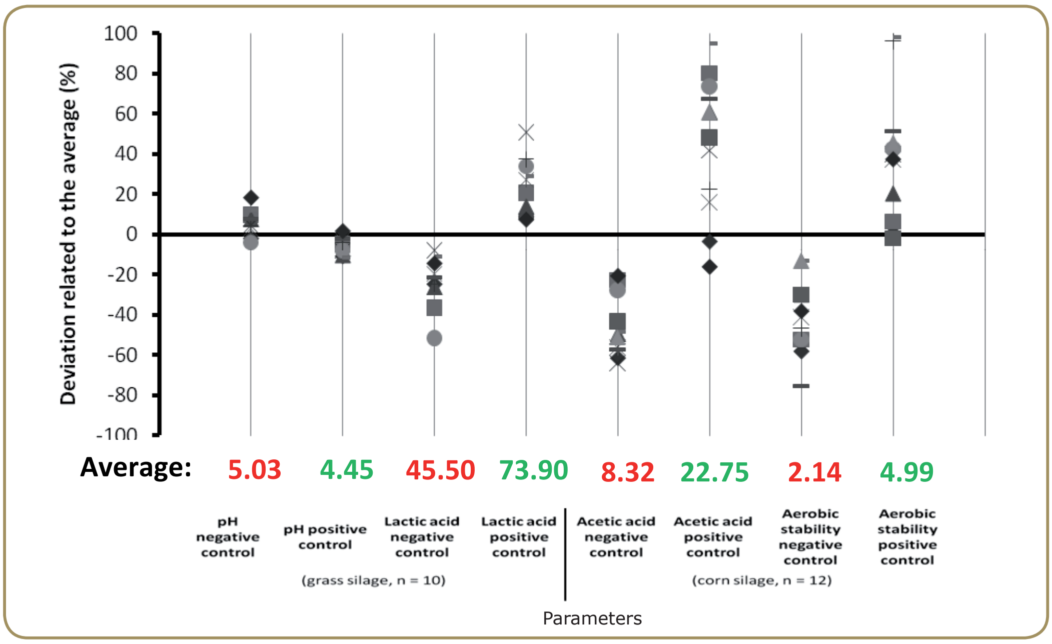
Picture 6: *Moulded silage*

The main parameters for controlling the growth of the above described microorganisms are summarized in *Table 2*.

THE CONTROL OF HARMFUL MICROORGANISMS PRESENT IN DEFICIENT SILAGES

The examples are based on the results obtained in field trials with silages inoculated with blends of homo- and heterofermentative bacteria (Biomin® BioStabil Plus - 20 grass silages and Biomin® BioStabil Mays - 24 corn silages). Different substrates were used to refer to the silage quality parameters. In this study, only the parameters that can be directly influenced by the use of silage inoculants were selected (pH value, lactic and acetic acid and aerobic stability).

The results of the trials conducted with silages that have and have not been treated with silage inoculants are presented in *Graph 1*. As shown in *Graph 1*, the use of a silage inoculant improves the fermentation and lactic acid production (on average, 0.58 and in 28.4g/kg of dry matter respectively) in grass silages. The use of a silage inoculant that contains heterofermentative lactic acid bacteria (*L. brevis*) improves the acetic acid production and the aerobic stability in corn silages in 14.43g/kg of dry matter (+173 %) and 2.85 days (+133 %) respectively.



Graph 1: *Influence of silage inoculants on selected parameters of the silage quality*

Conclusion

The use of silage inoculants can help to manage infection risks.

Combined with the absence of oxygen in the silage, a rapid and deep decrease in the pH value due to homofermentative lactic acid bacteria controls the growth of *Listeria monocytogenes*, *Clostridia* and *Enterobacteria*. The decrease in the pH value is closely related to a higher lactic acid production (r^2 = -0.80 to -0.90).

The increase in the acetic acid production due to the use of silage inoculants containing heterofermentative lactic acid bacteria increases the aerobic stability. The aerobic stability is measured through the increase in the silage temperature compared with the ambient temperature and influences the activity of yeasts. A longer aerobic stability means that the yeasts are unable to grow and produce exothermic reactions. The aerobic stability is measured through the increase in the silage temperature compared with the ambient temperature and causes considerable losses in nutrients and energy in the silage.

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