Minimising mycotoxins in silages

CORN SILAGES ARE USUALLY WELL FERMENTED, HOWEVER, AFTER OPENING, FUNGAL GROWTH IS A RECURRENT PROBLEM. FUNGUS CONTAMINATION IS NOT ONLY THE CAUSE OF SIGNIFICANT LOSSES, BUT ALSO THE SOURCE OF INVISIBLE ENEMIES OF CONCERN IN ANIMAL PRODUCTION AND HUMAN NUTRITION: MYCOTOXINS. ALEX BACH AND CRISTINA IGLESIAS INVESTIGATED THE EFFECTIVENESS OF A SILAGE INOCULANT IN REDUCING MYCOTOXINS IN CORN SILAGES.

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The FAO estimates that 25% of world food crops are affected by mycotoxins each year. The most common mycotoxins found in corn silages are DON (vomitoxin), zearalenone and aflatoxin. Aflatoxins are probably the best-known mycotoxins, due to their potent carcinogenic effects. Aflatoxin in feed is excreted in milk in the form of aflatoxin M1 and presents a human health hazard. The EU legislation establishes a maximum concentration of aflatoxin M1 in milk of 0.05ppb. Zearalenone exhibits estrogenic properties and has been shown to cause reproductive failure in dairy cattle (Khamis *et al*, 1986). Vomitoxin is also toxic at high doses (with effects on the liver and necrotic effects on skin and mucosa), although it seems to have little consequences in ruminants.

Lactobacillus buchneri NCIMB 40788, originally isolated from naturally occurring aerobically stable silages, was selected for its anti-fungal activity and developed as a silage inoculant (Lalsil ®Fresh, Lallemand, France) for high-sugar, high DM forages. It is especially recommended for whole crop corn silos opened during the hot season. Previous studies have shown that corn silages treated with *L. buchneri* NCIMB 40788 exhibit greater concentrations of acetic and propionic acids, and lower levels of lactic acid than untreated silages (Driehuis *et al*, 1999). Also, several studies (Driehuis *et al*, 1999; Kung and Ranjit, 2001; Adesogan *et al*, 2003) have shown the anti-fungal activity of *L. buchneri* NCIMB 40788 under laboratory conditions. However, no study has been carried out to evaluate the effectiveness of *L. buchneri* NCIMB 40788 on corn silages in field conditions, where the risk of microbial contamination is greater.

FIGURE 1 – FUNGAL CONTAMINATION 4 DAYS AFTER OPENING THE SILOS (AVERAGE COUNTS FROM THE 28 SILOS).

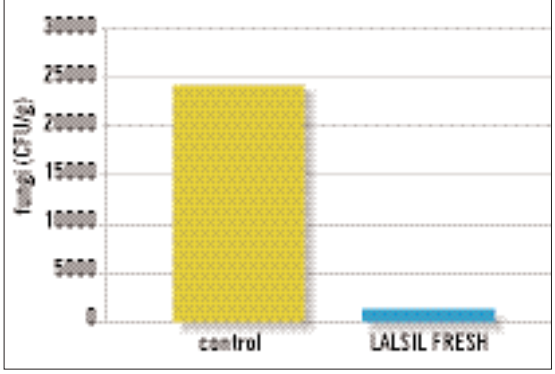
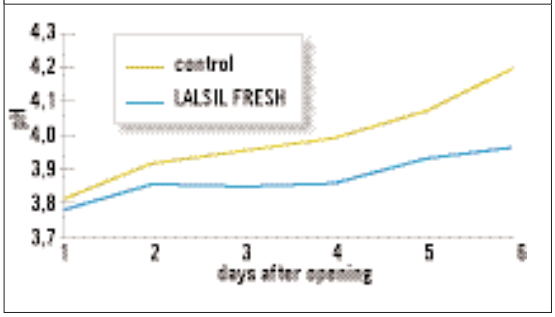


FIGURE 2 – EFFECT OF SILAGE INOCULANT ON PH AFTER OPENING.



Placement of control and L. buchneri NCIMB 40788-treated bags within the silage (left: before closing the silage; right: after opening the silage).

A FIELD STUDY

A study was conducted with the aim of testing the effect of *L. buchneri* NCIMB 40788 on corn silage stability and especially on mycotoxin contents, in field conditions. For this reason, a field study was done covering 28 commercial dairy herds in Girona (Spain), to include the variations in mycotoxin contents at harvest among fields and generate data applicable to normal farming conditions.

The farms were visited at the time of corn harvest. While corn was chopped, processed and packed into the silo, 10 kg of corn material were treated with 200 ml of water containing 10 g of freeze dried *Lactobacillus buchneri* NCIMB 40788, to obtain the minimal inoculation rate of 3.10^5 CFU/g fresh forage, and placed in a 10-kg bag. Using the same corn material, 10 kg were processed in the same fashion for the untreated control, but sprayed with water only, to add the same amount of moisture as in the treated material. Both bags were placed at the same level within the silo and covered with more fresh material (*see picture*). A yellow or green piece of plastic was placed about 60 cm in front of each bag, serving as a signal to know when to get ready to collect the samples for testing. Chemical composition, pH, and mycotoxin concentrations (aflatoxin, DON, and zearalenone) were determined for the 28 silos tested, before and after ensiling. To assess the conservation of the silages, after opening the sample bags, fungal counts (yeast excluded), volatile fatty acid profile, and lactic acid concentrations were determined. Then, the pH within each sample was monitored daily for a week after opening. To assess the aerobic stability of the silages, mycotoxin levels and fungal counts were determined four days after opening the silage.

FIGURE 3 – THE IMPACT OF L. BUCHNERI NCIMB 40788 ON VOLATILE FATTY ACID CONCENTRATIONS (MM) IN CORN SILAGES.

Compound	Control	Lalsil Fresh treated	P-value
VFA	246.98	311.93	0.031
Acetate	249.7	296.91	0.029
Propionate	14.98	14.49	NS
n-Butyrate	0.39	0.05	NS
Isobutyrate	0.18	0.42	0.08
n-Valerate	0.19	0.01	NS
Isovalerate	0.15	0.09	NS
Lactic acid	6.95	7.48	NS

IMPROVED SILAGE CONSERVATION

At the opening of the silos, fungal contamination was relatively low, both in treated and non-treated silages, showing a good conservation of the corn silages. However, silages treated with the inoculant showed a numerically lower fungal count at opening, and this difference increased as silages were further exposed to air (*Figure 1*). This confirms previous studies showing the anti-fungal activity of *L. buchneri* NCIMB 40788 (Adesogan *et al*, 2003).

The evolution of pH after opening a silo is another good indicator of the quality of conservation of silage. After opening, the pH was more stable in *L. buchneri* NCIMB 40788 treated silages than in control ones (*Figure 2*). Low pH prevents the growth of fungi and bacteria within the silos, helping to achieve good conservation.

Lactobacillus buchneri NCIMB 40788 is an heterolactic bacteria, able to ferment lactic acid into acetic acid and 1,2-propanediol under anaerobic conditions. Acetic acid is a potent inhibitor of fungi development (Weinberg *et al*, 1993). When comparing the chemical analysis of the silages before and after ensiling (*Table 1*), the treated silages showed a greater concentration of acetate and greater volatile fatty acid (VFA) concentrations(*Figure 3*). This was probably the cause of the observed low pH, since other acids (lactate, propionate, butyrate, valerate and isovalerate) did not differ between treatments. The concentration of isobutyrate tended to increase.

Additionally, the chemical composition of silages after fermentation was not significantly different between control and treated silos, showing that the differences in fermentation end-products were not a consequence of a different use of fermentation substrate. From this, it can be concluded that *L. buchneri* NCIMB 40788 did not change the nutritional quality of corn silages.

REDUCED MYCOTOXIN CONTENTS

Mycotoxins are the products of moulds, not yeast. This is the reason why yeast was not included in the total fungus count. However, the presence or absence of fungi in a silo cannot be used as sole indicator of mycotoxin contamination: there might be fungal growth without release of mycotoxins, or there might be mycotoxins without fungal presence, because fungi were present at some point, produced mycotoxins that accumulated within the silage, and later died (Wyatt, 1991). In this study the lev-

FIGURE 4 – EFFECT OF SILAGE INOCULANT ON SILAGE AFLATOXIN AFTER OPENING.

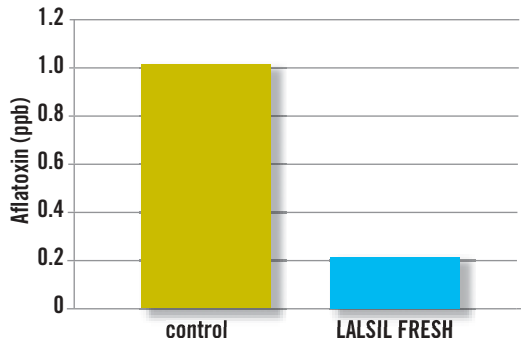
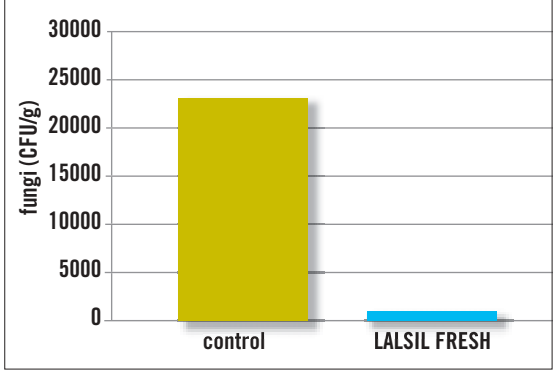


FIGURE 5 – EFFECT OF SILAGE INOCULANT ON SILAGE ZEARALENONE AFTER OPENING.



els of three main mycotoxins in corn silages were assessed: aflatoxin, zearalenone, and vomitoxin.

Before ensiling, the average aflatoxin content of fresh corn was 7.35 ppb, probably a consequence of the presence of fungi in the fields, a parameter that cannot be easily controlled. It is interesting to notice that, after ensiling, aflatoxin concentrations significantly decreased, showing a detoxifying property apparently inherent to the ensiling process. Moreover, the silages treated with *L. buchneri* NCIMB 40788 tended (P = 0.08) to show lower contents of aflatoxin than control silages (*Figure 4*), suggesting some direct or indirect effects of the inoculant on aflatoxin reduction.

Before ensiling, the average zearalenone content of corn was 75.16 ppb. Contrary to the observations with aflatoxin, zearalenone concentration increased after ensiling, although the inoculant treatment numerically limited this increase during the ensiling process (*Figure 5*).

Finally, fresh corn had an average DON (Vomitoxin) content of 0.04 ppm, which increased to 0.27 ppm after ensiling. There were no significant differences in DON concentrations after ensiling between the control and treated silos.

Despite the relatively low fungal counts, four days after opening the silages and leaving them exposed to oxygen, the mycotoxin contents of silages increased dramatically (data not shown). However, the *L. buchneri* NCIMB 40788 treated silages had numerically lower contents of all mycotoxins tested than the untreated ones.

In conclusion, the numerically lower fungal counts, and the significant greater concentrations of acetic acid linked to the treatment of the silage resulted in numerically lower concentrations of zearalenone and DON, and tended to have lower aflatoxin concentrations four days after opening the silages.

NEW PERSPECTIVES FOR CORN SILAGES

It is usually difficult to control mycotoxin contamination in silages. It depends first of all on the initial fungus and thus mycotoxin contents at harvest, which can differ greatly depending on the climate, the type of crop, the environment, and so forth, and can be difficult to control by producers. On the other hand, it also depends on the growth of fungi within the silos after ensiling. The most common way of limiting this second level of contamination is by implementing good ensiling practices, but no technical aides were available. The field trials conducted by IRTA proved that *L. buchneri* NCIMB 40788 may help in limiting the levels of certain mycotoxins in corn silages. This, in combination with good ensiling practice, can be a useful tool for producers, helping to limit the impact of mycotoxins on animal and human health.

Finally, it is interesting to highlight the apparent detoxifying effect inherent to the corn ensiling process for aflatoxin contamination in corn silos, which is a strong argument in favour of corn silage. <-

References are available on request.

Dr Alex Bach obtained a veterinary degree from the Universitat Autònoma de Barcelona in 1994, before pursuing a Masters and a PhD in dairy science at the University of Minnesota. He was responsible for the dairy research programme of the European branch of a multinational feed company for three years, and has been directing the Ruminant Unit of IRTA (Institute of Agrifood Research and Technology), in Barcelona, Spain since 2002. Dr Bach is author or co-author of more than 20 scientific publications on dairy cattle nutrition and management, with more than 30 extension papers, and he has also participated in writing four books or book chapters on dairy management and nutrition.