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E. Kaiser

*The Institute of Animal Science, Germany*

K. Weiß

*The Institute of Animal Science, Germany*

I. Polip

*The Institute of Animal Science, Germany*

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## New results on inhibition of clostridia development in silages

E. Kaiser, K. Weiß and I. Polip

The Institute of Animal Science, Humboldt-University of Berlin, Invalidenstraße 42, D-10115 Berlin, Germany

Email: ehrengard.kaiser@agrar.hu-berlin.de

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**Introduction** The prevention of clostridial activity in silages is one of the most important aims in silage making. Clostridial activity in silages is especially expressed as the occurrence of butyric acid and as increased content of clostridial spores. A rapid reduction in the pH value at the beginning of fermentation process is considered as the most important factor for inhibition of clostridial development. It is assumed, that, if the “critical pH value” will be quickly achieved, clostridial activity in silages can be stopped. In experiments concerning the fermentation process it was found that the effect of acidification and dry matter content on the clostridial activity is different in ensiling material, containing nitrate, and in nitrate-free material. The object of the present paper was to clarify the conditions for clostridial development during the fermentation process, including examination of factors such as dry matter content, acidification and nitrate content.

**Materials and methods** The fermentation process in *Dactylis glomerata*, first growth and nitrate-free, was examined at four levels of dry matter (DM) content (217 g/kg, 325 g/kg, 416 g/kg, 515 g/kg) of the ensiling material under laboratory conditions, in each case without and with additives. To improve the acidification, lactic acid bacteria (LAB) was used in a concentration of  $10^5$  cfu g/FM and LAB ( $10^5$  cfu g/FM) + glucose (2% of fresh matter) respectively. The additive of nitrate was 4.4 as well as 6.6 g  $\text{NO}_3/\text{kg}$  DM. The silages were analysed after 3, 7, 14, 28, 56 and 180 days of storage period under temperature constant conditions (25°C). To determine effects of DM, acidification and nitrate on clostridia development the ensiling material was contaminated with additives of clostridia spores (approximately  $10^4$  MPN g/FM).

**Results** Although a rapid setting of the critical pH value was achieved, significant butyric acid (BA) formation was observed in silages, which was earlier (on DM of 217 g/kg from d 7) and more comprehensive with lower DM content (Table 1). Butyric acid-free silages were only estimated in the fermentation process of ensiling material with the level of 515 g/kg DM. At lower levels of DM, the BA formation was only restricted, but not suppressed by acidification. In contrast the formation of BA was also suppressed at lower levels of DM by nitrate additive. The addition of LAB and LAB + glucose had no positive effect on the development of clostridia spores at lower DM levels 217 (Table 1) and 325 g/kg. Indeed the content of clostridia spores decreased after commencement of fermentation. The content of clostridia spores increased with commencement of butyric acid formation and reached an extent, which is higher than the content of ensiling material. With nitrate as an additive (Table 1) the clostridia spore content was reduced to nearly zero at two levels of DM. In contrast at high DM contents the effects on clostridia have been observed, whereas the effect of nitrate was not so clearly pronounced.

**Table 1** Effects of DM content, acidification and nitrate on clostridia development during fermentation process

	After 56 days of storage period (DM g/kg)				After 14 days of storage period (DM content 217 g/kg)		
	217	325	416	515	Control	LAB	Nitrate
pH	4.3	4.4	4.6	4.6	4.4	3.6	4.
BA (g/kg)	36	16	12	0	14	6	0
Clostridia spores (MPN g/FM)	$2.3 \times 10^6$	$6.8 \times 10^5$	$2.0 \times 10^5$	$9.3 \times 10^3$	$2.5 \times 10^6$	$1.2 \times 10^4$	$1.8 \times 10^3$

**Conclusions** The decrease in pH value is not the major inhibiting parameter against clostridia activity. At low DM, a directly acting clostridia inhibitor is necessary in addition to acidification. At high DM content the reduced availability of water (decreased  $a_w$ -value) is effective as an inhibiting parameter, whereas the importance of acidification decreased. Acidification,  $a_w$ -value and nitrate complement are inhibiting factors for clostridia development. This synergistic effect is very different at varying DM levels.

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