

Effect of inoculation on silage quality and rumen fermentation in dairy cows

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Summary

The purpose of the study was to determine the effect of a silage additive on maize silage fermentation and assess the relationship of silages with different levels of fermentation end-products to ruminal fermentation parameters and organic matter (OM) digestibility. The maize silage underwent two treatments: a) no additive (control silage), b) additive with *Lactobacillus plantarum* and *Pediococcus acidilactici* and enzyme cellulase. The additive improved silage fermentative processes, preserved more water soluble carbohydrates, increased fermentation of lactic acid and decreased formation of ammonia-N. Two groups of analogous cows were formed: the cows (n = 10) of the control group were fed silage without additives, the cows (n = 10) of the experimental group – the silage additive. Rumen fluid from cows was sampled. The silage additive improved fermentation processes in the rumen: the amount of volatile fatty acids, including propionic acid, increased as well as the total and lactate fermenting bacterial count and number of protozoa. The incubation of both grass hay and also silage with the rumen fluid from the cows receiving silage with inoculant and cellulase additive improved their OM digestibility.

Keywords: silage, rumen

Formation of microbial ecosystem in the rumen is considered to be quite a complicated process and depends on many factors (14, 19). Forage quality, method of preparation and ration composition directly effect these processes (13).

When forages are preserved as silage, water soluble carbohydrates (WSC) of the grass or other plants are fermented to lactic acid and volatile fatty acids (VFA). The extent of fermentation of WSC during the ensilaging can change proportions of the end-products of rumen fermentation. The majority of published reports indicate propionate as the main end-product of lactate fermentation on the silage based diets (10). Diets based on restrictively fermented grass silages which are high in WSC and low in lactate, favourably effect a rumen fermentation pattern rich in butyrate or acetate and low in propionate. Silages low in WSC and high in lactic acid tend to increase the proportion of propionate in ruminal fluid. According to Cushnahan et al. (7) silages treated with inoculants appeared to be more digestible than untreated silages. It seems that the ingestion of silage fermentation end-products may modify rumen fermentation patterns and nutrient digestion.

In order to ensure good health of cattle, production increase and its improved quality, bacterial species *Lac-*

tobacillus plantarum and *Pediococcus acidilactici* seem to be of primary important (5, 18). In recent years many studies have explored the possibility of improving the digestibility of dietary fibre by using exogenous cell wall-degrading enzymes, such as cellulase (2). Giraldo et al. (8) found that the cellulase treatment increased acetate, propionate and total VFA production, as well as neutral detergent fibre degradability.

A study was carried out to determine the effects of a biological silage preparation on the extent of maize silage fermentation and assess the relationship of silages with different levels of fermentation end-products to ruminal fermentation parameters and organic matter (OM) digestibility.

Material and methods

The silages were produced from whole maize plants. Impact variety in the third decade of September. The ensiled material contained 241 g DM kg⁻¹ and 89 g crude protein, 107 g WSC kg⁻¹ DM. Silage were underwent two treatments: a) no additive (control silage), b) additive of *Lactobacillus plantarum* (NCIB 300083;30084) and *Pediococcus acidilactici* (NCIB 300085; 30086) and enzyme cellulase. The target level of the silage additive was 10⁶ cfu g⁻¹ maize.

Silage DM content was determined by oven drying at 67°C for 24 h, equilibrated to room humidity overnight, milled

through a 1.00 mm sieve and further dried at 105°C to a constant weight. The standard methods according to AOAC (1), for basic nutrient determination in the feed were used.

WSC were estimated by the anthrone method of Thomas (24). Lactic acid, VFA were determined in an aqueous extract for fresh silage according to the standard methods (17). The pH value of silages was determined electrometrically using a pH-meter.

The experiment was carried out during the indoor period. Experimental cows ($n = 20$) 500-550 kg body weight were kept tied, with the possibility of 2 hours of motion daily, and fed individually with free access to water. They were milked twice daily. The animals were kept in a 4-week preliminary period when for all 20 cows control silage was offered *ad libitum* in two respective portions per day. A concentrate mixture (consisting of barley (75%); wheat (10%); soybean meal (12%) and mineral-vitamin supplement (3%)) was fed individually according to milk yield twice daily.

After grouping the animals according to the principle of analogy, taking into consideration age of cows, body weight, number of lactations and daily milk yield, two groups of cows were formed. Each group was fed the respective silage *ad libitum* offered twice daily. The cows ($n = 10$) of group C (control) were fed control silage without additives, the cows ($n = 10$) of group E (experimental) – the silage additive. Concentrate mixture for the cows of both groups was offered in the same way as during preliminary period.

Rumen fluid was sampled by a throat-esophagus tube GDZ-1 (21) from the caudoventral part of the rumen, 3 hours after morning feeding. The rumen fluid pH was measured by an electrometric method using a pH-meter CP-315 (23). Protozoa were counted in 1 ml of the rumen fluid in Fuks-Rozental chamber (23). The total amount of VFA was ascertained by distilling the rumen fluid in Markgamus apparatus (22) and after that by using a gaschromatograph GC-2010 Shimadzu (Japan). The percentage ratio (molar) of particular acids in their sum was calculated. Total bacterial count (TBC), lactate fermenting bacterial count (LFBC) and cellulolytic bacterial count (CBC) were studied in the rumen fluid of cows according to the method suggested by Van Gylswyk (25) for cultivating obligate anaerobes.

Organic matter digestibility (OMD) of forage was studied by the *in vitro* method (15). During these investigations all samples of forages were incubated with the rumen fluid of each individual cow. The essence of the experiment is that forage is incubated with rumen fluid for 96 hours under strictly anaerobic condition in special glass tubes at 39°C temperature in a water bath.

The data of the experiments were evaluated statistically by a statistic package R 2.2.0. (26). The reliability of the differences between arithmetic means (p) was defined according to the Student t-test (11). The results are considered to be reliable when $p < 0.05$, $p < 0.01$.

Results and discussion

Silage enriched with inoculant and cellulase contained significantly more lactic acid ($p < 0.01$) and less ammonia-N ($p < 0.01$) when compared to the untreated maize silage (fig. 1). Additives in silage prevented butyric acid formation. Treatment by lactic-acid-producing bacteria preserved more WSC ($p < 0.01$) in the silage.

Biochemical parameters were defined during the course of studying the rumen fluid that increased in the experimental group of cows. The rumen fluid pH corresponded to the physiological norms for cattle (22) and it was

slightly increased (by 0.08 pH). It was found that the number of protozoa in the rumen fluid of group E cows increased by 0.06 log/ml ($p < 0.05$) compared to the cows of the control group. The obtained results confirm the data of other authors that the total protozoa count in the rumen fluid of cows directly depends on forage preparation technologies ration composition and structure (16). According to Betliev (4) dry matter loss during fermentation is lower due to a more direct process of fermentation in the silage with bacterial inoculant additive. The resultant silage contains higher amounts of metabolically potent energy and protein. In the case when cows of group E were fed such silage, the protozoa count increased, as well as carbohydrate assimilation and the overall animal nutrition improved.

When the cows of group E were fed with inoculated silage, the production of VFA in the rumen increased by 6.12 mmol/l ($p < 0.05$) compared to the control group (tab. 1). Besong et al. (3) indicates that high amounts of silage and concentrates in cattle feed leads to the higher production of VFA. The major part of sugar in silaged raw substances ferments into organic acids, however, the amount of WSC in the inoculated silage tended to be higher. Giraldo et al. (8) states, that scarcity of carbohydrates in the forage leads to a reduced production of organic acids which consequently causes a shortage of energy required by the animal.

The portion of propionic acid in the total rumen VFA of group E cows increased by 0.8 percentage unit ($p <$

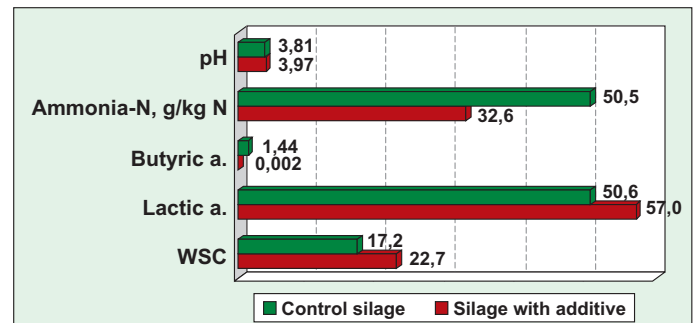


Fig. 1. Effect of inoculation on silage pH and contents of some components (g/kg DM)

Tab. 1. Biochemical and microbiological parameters in the rumen of cows

Parameters	Control group (n = 10)	Experimental group (n = 10)	P
pH	6.11 ± 0.04	6.19 ± 0.04	> 0.05
Total VFA, mmol/l	106.28 ± 2.58	112.40 ± 1.08	< 0.05
%mol:			
acetic acid	60.56 ± 0.74	60.64 ± 0.28	> 0.05
propionic acid	17.68 ± 0.26	18.48 ± 0.29	< 0.05
butyric acid	13.83 ± 0.54	14.17 ± 0.24	> 0.05
Number of protozoa, log/ml	5.42 ± 0.02	5.48 ± 0.01	< 0.05
TBC, log/ml	11.54 ± 0.02	11.59 ± 0.02	< 0.05
LFBC, log/ml	8.72 ± 0.01	8.76 ± 0.01	< 0.05
CBC, log/ml	6.61 ± 0.01	6.59 ± 0.02	> 0.05

0.05). In cases where forage contains low amount of fiber and high amounts of easily degradable carbohydrates, especially starch, the production of propionic acid in the rumen is quite effective (up to 40% of total rumen VFA), (3). Products of starch degradation are effectively used for milk synthesis and as the source of energy for rumen microflora and matabolization processes in the organism (6, 9). Consequently, when feed is devised, attention should be paid to the ratio of easily and heavily degradable starch in the forage. When animals are fed forages with high amounts of easily degradable starch, it can cause high production of organic acids and development of acidosis (12).

In group E of cows, given inoculated silage, the total bacterial count in the rumen increased by 0.05 log/ml ($p < 0.05$), LFBC was by 0.04 log/ml higher ($p < 0.05$), in comparison with control (tab. 1). This was probably mainly due to the direct effect of the inoculant of lactic acid bacteria added to the ensiled maize. However, part of this effect could be also due to the cellulase introduced to the ensiled material. According to Van Gylswyk (25) the enzyme cellulase is able to degrade plant cell walls, release sugar from complex carbohydrate compounds and create optimal conditions for lactic acid bacteria to produce lactic acid. Effective propagations of this bacterial species leads to a higher number of lactate fermenting bacteria ($p < 0.05$) due to the fact that lactic acid in the rumen is not only produced, but fermented as well.

The investigation of forage OM digestibility *in vitro* led to the conclusion that the OM digestibility of hay from cultural grasses and pastures was by 0.89 percentage unit ($p < 0.05$), and silage – by 2.49 percentage unit higher ($p < 0.05$), in the case of incubating with the rumen fluid taken from the experimental group compared with the control (fig. 2). A crucial role in this increase of OM digestibility could have the cellulase additive to the ensiled maize. The cellulase preparation is able to degrade plant cell walls and stimulate fiber fermentation by cellulytic bacteria (27) and consequently organic matter digestibility (10).

The inoculant improved silage fermentative processes, preserved more water soluble carbohydrates, increased fermentation of lactic acid and decreased formation of ammonia-N.

The amount of VFA increased in the rumen of the cows which were fed silage containing inoculant and cellalase. An increase was also noted in the total and lactate fermenting bacterial count, amount of propionic acid and number of protozoa in the rumen. The incubation of both grass hay and silage with the rumen fluid from cows receiving silage containing inoculant and cellulase additives improved their OM digestibility.

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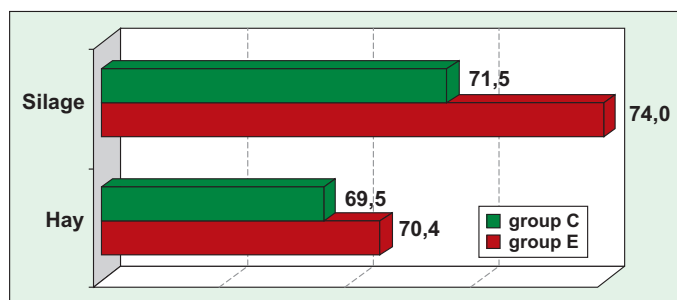


Fig. 2. *In vitro* organic matter digestibility of silage and hay, incubated in different rumen fluids

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