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Effect of Microbial Inoculants on the Nutritive Value of Corn Silage for Beef Cattle

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Abstract: This study investigated the effect of a new microbial inoculant product on the composition and nutritive value of corn silage in big silo over one year that used beef cattle. Six Holstein beef steer (BW = 225±17) were allotted to 2×2 repeated Latin square design at two 21 days periods (adaptation, 14 days and sample collection, 7 days) for evaluation the effect of microbial inoculation on the composition and nutritive value of corn silage for beef cattle. Two treatments, forages were untreated or treated at ensiling with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculants. After 45 days from ensiling, the ration that contained 94.5 and 5, 0.2, 0.2, 0.1% of DM silage and ground barely, mineral-vitamin, dicalcium phosphate, salt, respectively, were offered for free choice consumption. Treatment with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* inoculant increased daily dry matter intake and subsequently NDF, ether extract, crude protein and ash. Apparent digestibility of DM and nutrients were significantly increased by microbial inoculation. Microbial inoculation can improve the nutritive value of corn silage for beef cattle.

Key words: Silage, microbial inoculant, nutritive value, beef cattle

INTRODUCTION

Normally during ensiling the fodder undergoes an acid fermentation in which bacteria produce lactic, acetic and butyric acids from Water Soluble Carbohydrates (WSC) present in the raw material. The net result is a reduction in pH, which prevents the growth of spoilage microorganisms (McDonald *et al*., 1991). In order to reduce the dependence of the ensiling process on epiphytic Lactic Acid Bacteria (LAB) and on chemical additives, inoculants containing selected strains of LAB have been developed. Inoculants, comprising homofermentative bacteria such as *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus* species, are often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH. The function of these inoculants is to ensure a rapid and efficient fermentation of WSC into lactic acid by homolactic fermentation. These results in an intensive build-up of lactic acid, rapid decrease in pH and improved silage preservation with minimal fermentation losses (Filya, 2004; Filya *et al*., 2004). Gordon (1989) and Kung *et al.* (1993) found that microbial inoculation has improved the fermentation of grass and legume silages. Microbial inoculants are applied to forages at the time of ensiling to accelerate the decline of pH during the initial stage of silage fermentation, to preserve plant carbohydrates through homofermentation and to preserve plant protein by decreasing proteolysis and deamination (Seale, 1986). Thus, inoculated silages are expected to improve animal performance (McDonald *et al*., 1991). Muck and Kung (1997) reported that microbial inoculation lowered pH, improved the lactic: acetic ratio and lowered ammonia nitrogen content in more than 60% of studies. Dry matter recovery was improved by more than 35% and bunk life improved in about 30% of the studies. Dry matter digestibility was also improved in about one third of the cases. Microbial inoculation usually has little or no effect on the fiber content of silages because most lactic acid bacteria contain little or no ability to degrade plant cell walls. Decreases in fiber content may be due to partial acid hydrolysis of hemicellulose. Some data suggests that certain microbial inoculants can increase fiber digestion (Rice *et al*., 1990). Bunk life or aerobic stability has not been consistently improved by inoculation and in some instance inoculation has made aerobic stability worse. This is probably due to lower acetic acid content. However, changes in silage fermentation have not always been related to improved animal performance. However, It is possible to apply bacterial inoculants at ensiling in order to promote adequate fermentation patterns.

The inoculated silages appeared to be more stable upon exposure to air. Air is detrimental to silage quality because it enables aerobic spoilage microorganisms, such as yeasts and molds, to become active (Woolford, 1990).

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During exposure to air during the feedout phase, silage also might undergo increases in temperature and pH and losses of WSC and fermentation end products, which reduce silage quality and digestibility (Pitt *et al*., 1991). Susceptibility to spoilage is especially a problem in warm climates and is a very important factor in determining silage quality and value (Ashbell *et al*., 2002). Therefore, under warm conditions, additives that protect the silage upon exposure to air might be very useful.

The objective of this study was to determine the effect of microbial inoculation on the fermentation of corn silage treated with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculants and the subsequent effect on nutritive value and performance by lactating beef cattle.

MATERIALS AND METHODS

Silage preparation: At 23 August in 2006, whole plant corn was harvested at medium dough stage of maturity (24.2% DM) and used for ensilaging. Forage was chopped by a conventional forage harvester and was ensiled in horizontal silos directly after harvest. In two big silos that had capacity more than 50 tones, silages were prepared without inoculants (control) or with one of commercially available inoculants (LALSIL MS01, Lallemand, France). The inoculants was composed of a strain of lactic bacteria (*Lactobacillus plantarum* MA 18.5U) and a strain of propionic bacteria (*Propionibacterium acidipropionici* MA26/4U). The application rate determined by the manufacturers stated the level of LAB in the products. Inoculants were dissolved in tap water and applied according to the label instructions for application (0.0005%). The inoculants were diluted in tap water and applied at the rate of 2 mL kg^{-1} of forage with a sprayer. Approximately 45 to 55 tones of forage were made for each treatment. All forages were treated and sealed in horizontal silos within 6 h on a clear day. All inoculants were applied to the forages in a uniform manner with constant mixing. After 30 days of ensiling, each silo was opened and samples were collected and their pH was measured directly on the silage juice, using a pH meter. Samples of silage were dried at 55°C for 48 h in a forced-air oven to constant weight for DM, ground through a Wiley mill (1 mm screen pore size), analyzed for DM, crud protein, ether extract and ash at 550°C at 3 h (AOAC, 2002), NDF (Van Soest *et al*., 1991; Table 1). Non fibrous carbohydrate was calculated by 100-(CP%+NDF%+ Ash%+EE%) (National Research Council, 2001) (Table 1). The concentration of N-NH_3 was measured with Kjeltec Auto Analyzer (Model 1030, Tecator Co. Sweden; Table 1). Chemical analyses were performed in triplicate.

Table 1: pH and chemical composition of silage

Item	Silage	
	Uninoculated	Inoculated
pH of corn cub before ensiling	6.23	6.23
NH_3-N concentration (mg dL^{-1})	8.96[a]	6.25[b]
pH of silage	4.22[a]	3.93[b]
Dry matter	24.55	24.15
Crnde protein	8.89	8.82
Neutral detergent fiber	62.22[a]	58.32[b]
Non fiber carbohydrate	19.43[b]	23.70[a]
Ether extract	3.20	3.11
Ash	5.98	6.33

Means within a row with different subscripts different at ($p<0.05$)

Table 2: Ingredients and chemical compositions of ration

Item	Proportion (% of DM)
Ingredients	
Silage	94.50
Barley	5.00
Mineral-vitamin	0.20
Dicalcium phosphate	0.20
Salt	0.10
Chemical composition (%)	
Dry matter	34.00
Crnde protein	10.51
Neutral detergent fiber	52.72
Non fiber carbohydrate	29.99
Ether extract	2.89
Ash	3.89

***In vivo* digestibility trial:** Six Holstein beef steer (BW = 225±17) were allotted to 2×2 repeated Latin square design at two 21 days periods (adaptation, 14 days and sample collection, 7 days) for evaluation the effect of microbial inoculation on the composition and nutritive value of corn silage. Diets had a similar composition and contained 94.5, 5.0, 0.2, 0.2 and 0.1, silage, barely, Dicalcium phosphate, mineral-vitamin mix supplement and salt, respectively (Table 2). Diets were formulated using the National Research Council (2001) to supply adequate NEm and protein for a 225 kg dairy steer at maintenance level. Diets had similar chemical composition (Table 2), but varied in particle silage. Water was available for steers over the experiment. Steers were housed in tie stalls and fed *ad libitum*, twice daily at 600 and 1800 h, allowing for at least 10% orts (as-fed basis). At collection period, feed, orts and waste were weighed; sub sampled and stored at -20°C. Steers had *ad libitum* access to feed for the first 14 days of diet adaptation and were subsequently fed at 95% of *ad libitum* throughout collection periods. Daily samples of forage, rations and orts were collected, dried at 55°C, ground through a Wiley mill (1 mm screen) and composited by animal within a period. Total feces were collected from all cows for 7 days of each period (14 to 21 days). Feces were dried at 55°C and ground through a Wiley mill (1 mm screen). Feed, feces and orts were analyzed for DM, OM, Kjeldahl N, ether extract, ash at 605°C (AOAC, 2002), NDF and ADF (Van Soest *et al*.,

Table 3: Effect of microbial inoculants on intake and nutrients digestibility of corn silage-based diets fed to beef steers

Item	Silage	
	Uninoculated	Inoculated
Intake (kg day^{-1})		
Dry matter	8.82^{b}	11.04^{a}
Crnde protein	1.10^{b}	1.40^{a}
Neutral detergent fiber	5.63^{b}	5.81^{a}
Non fiber carbohydrate	2.56^{b}	3.20^{a}
Ether extract	0.55^{b}	0.65^{a}
Ash	0.26^{b}	0.33^{a}
Digestibility (%)		
Dry matter	75.03^{b}	79.91^{a}
Crnde protein	72.30^{b}	76.35^{a}
Neutral detergent fiber	62.50^{b}	70.56^{a}
Non fiber carbohydrate	85.66^{b}	89.68^{a}
Ether extract	66.71^{b}	83.26^{a}
Ash	48.08^{b}	65.37^{a}

Means within a row with different subscripts different at ($p<0.05$)

1991). Non fibrous carbohydrate was calculated by 100-(CP%+NDF%+Ash%+EE%) (National Research Council, 2001; Table 2 and 3). Using the chemical components of rations and feces, intake and digestibility of nutrients were calculated (Table 3).

Statistical analyses: Using the PROC GLM of SAS (2002), the experimental data were analyzed as a 2×2 replicated Latin square design by following model:

$$Y_{ijkln} = \mu+T_i+S_j+cow_{k(j)}+period_{j(l)}+e_{ijkln}$$

where, Y_{ijkln} was the dependent variable; μ is the overall mean, T_i, is the random effect of the silage with or without microbial inoculation as treatments (i = 1 and 2); S_j, is the random effect of $_j$th square (j = 1 and 2); $cow_{k(j)}$, is the cow effect inside of each square; $period_{j(l)}$, is the effect of each period inside each square and e_{ijkln}, is experimental error. Means were separated using LSD with an alpha level $p>0.05$.

RESULTS AND DISCUSSION

The initial pH of all substrates that used for ensiling was similar. Inoculation of silage significantly reduced pH of silage. It was expected that LAB increase the fermentation rate, causing the pH to decline faster and lower. By using LAB inoculants products of fermentation are shifted, resulting in more lactic acid and less acetic acid, ethanol and carbon dioxide (Filya, 2003). Inoculation of silage significantly decreased the concentrations of ammonia-N of corn silages compared with the control silage (Table 1). McDonald *et al.* (1991) reported that lower pH values inhibited protein degradation insilages. Therefore, concentrations of ammonia-N of all corn silages were low in the experiment. The dry matter content of silage was no significantly different, but the dry matter content of control silage was higher than that of inoculated silage that these findings were similar to those reported by Kung *et al.* (1993). After 30 days of ensiling, inoculated silages had lower pH and fiber carbohydrate content. These results were confirmed with Filya (2003) and Filya *et al.* (2004). The NDF content of inoculated silage was significantly lowered than control silage that is result of more fermentation of NDF content of silage in inoculated silage. In addition, the lower NDF content of inoculated silage is result of partial acid hydrolysis of hemicelluloses (Muck and Kung, 1997). These results were confirmed with Kung and Ranjit (2001). Non fiber carbohydrate content of inoculated silage was significantly higher than control silage because inoculation may decrease storage losses because in most experiments adding an inoculant to hay crop silages increased rate of pH decline and increased lactic acid concentration in the silage. Seale (1986), in his review on bacterial inoculants for silages, reported that suitable fast acid producing strains in sufficient numbers might be as effective as silage additives if the dry matter and water soluble carbohydrate of the crop are high enough. In the present study, all silages had lower pH values at an earlier stage of ensiling (Table 1). A lower pH in high moisture silage was expected because of higher concentrations of water soluble carbohydrate and more extensive fermentation (McDonald *et al.*, 1991). Filya (2004) and Filya *et al.* (2004) concluded that extensive fermentation in low dry matter corn silages made at the milk stage led to higher fermentation losses. The same trend was shown in this experiment. Silage inoculants are additives containing LAB that can enhance fermentation, increases the rate and efficiency of fermentation, causing the pH to decline faster and consequently, is slightly decreased dry matter losses and protein soubilization can be reduced. However, the success of a bacterial inoculant as a silage additive depends on many factors, such as the type and properties of the crops to be ensiled, climatic conditions, epiphytic microflora, ensiling technique and the properties of the inoculant (McAllister *et al.*, 1995). However, Bolsen *et al.* (1989) concluded that whole crop corn was fermented rapidly and that bacterial inoculants had little effect on the rate and efficiency of silage fermentation. Observations reported by other researchers (Buchanan-Smith and Yao (1981) and Moon *et al.* (1981) were similar and the present results further confirm these earlier conclusions.

Ingredients and chemical compositions of ration that fed to steers are shown in Table 2. Effect of microbial inoculants on dry matter intake and nutrients digestibility of corn silage-based diets fed to beef steers is shown in Table 3.

The DMI was greater for steers consuming inoculated silage (11.04 kg day^{-1}) than for those consuming control silage (8.82 kg day^{-1}). These results were similar to those reported by Muck and Kung (1997), however, many researchers found that feeding microbial inoculated silage to cattle does not affect DMI, compared with feeding uninoculated silage (El Hag *et al*., 1982; Hinds *et al*., 1985; Luther, 1986). Accompanying the increased DMI by steers consuming inoculated silage were significant increases in NDF, CP, ether extract and Ash digestibility compared with steers fed control silage. Nutrient digestion was different in steers fed untreated or treated corn silage. Inoculated silage had significantly higher digestibility coefficients for all nutrients (Table 3). A significant increase in digestibility of DM, NDF, CP, ash and ether extract was the result of increased surface area available for microbial attack, ultimately resulting in a more rapid rate of ruminal fermentation and increased intake (Mertens, 1997). Partial digestion of the fibrous components of silage during ensiling may alter ruminal digestibility. Many experiments have shown that lactic acid bacteria-based inoculants have the potential to improve silage fermentation (McAllister *et al*., 1995; Moshtaghi Nia and Wittenberg, 1999), digestibility of silage and nutrient intake and average daily gain by cattle (McAllister *et al*., 1995). Recent research demonstrated that digestibility may be greater in grass-legume, legume, or corn silages treated with microbial inoculants as silage additives (Cleale *et al*., 1990; Kung *et al*., 1993). The effects of inoculants on digestibility may be a consequence of improved nutrient preservation during the fermentation process and conservation of a greater proportion of digestible nutrients (McDonald *et al*., 1991). Filya (2004) hypothesized that homofermentative LAB inoculants produced mainly lactic acid, which could serve as a substrate for lactate-assimilating yeasts upon aerobic exposure. Thus, only small amounts of short chain volatile fatty acids such as acetic, propionic and butyric acids are produced. These VFA can inhibit yeasts and molds, making silages treated with homofermentative LAB inoculants deteriorate faster upon exposure to air. However, Filya *et al*. (2000) reported that the presence of low concentrations of oxygen in silage results in a shift of homolactic fermentation to heterolactic metabolism, leading to production of VFA, which possess antimycotic activity and inhibit the development of yeasts and molds.

CONCLUSIONS

Addition of microbial inoculants to corn silage caused improve in the nutritive value of corn silage for beef cattle. Inoculation of silage with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculant had effects on the nutritive value of corn silage. Inoculation with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculant tended to decrease pH and increase DMI and nutrient digestibility in the experiment. Inoculation with homofermentative LAB to dominate the epiphytic bacteria can increase fermentation efficiency but is less efficient if fermentable substrate is insufficient.

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