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Effects of *Lactobacillus buchneri* inoculation or 1-propanol supplementation to corn silage on the performance of lactating Holstein cows

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ABSTRACT - The objective was to evaluate the effects of corn silage supplemented with 1-propanol or inoculated with *L. buchneri* on the ruminal fermentation profile, digestibility, and production traits of lactating Holstein cows. Whole-corn plants were harvested at 350 g/kg dry matter (DM) and packed in nine bag silos (13 t/silo). At ensiling, two treatments were applied: control (no additive; six silos) and *L. buchneri* inoculation with 1×10^5 cfu/g (three silos). Feeding started after 247 days of storage; one *L. buchneri* and two control silos were opened in each experimental period. Twenty-one multiparous Holstein cows (~33 kg/day of milk) were allocated to seven balanced 3 × 3 Latin squares with 21-day periods (14 days of adaptation). The experimental diets contained (DM basis): 80 g/kg cottonseed, 95 g/kg citrus pulp, 180 g/kg soybean meal, 90 g/kg corn grain (ground), 25 g/kg minerals and vitamins premix, and 530 g/kg of corn silage. Source of corn silage was the only difference between experimental treatments: control, *L. buchneri*, or control silage supplemented with 1-propanol (10 g/kg of diet DM). The 1-propanol was dissolved in water (1:1) and sprinkled onto the ration during mixing, immediately before each feeding. Dry matter intake, nutrient digestibility, milk yield, and composition were not affected by treatments. Cows fed 1-propanol had greater concentrations of 1-propanol in the rumen fluid, higher concentration of glucose, and lower concentration of non-esterified fatty acids in blood plasma. Corn silage inoculated with *L. buchneri* at 1×10^5 cfu/g does not affect silage fermentation, ruminal fermentation profile, or milk production. Supplementation of 1-propanol at 10 g/kg affects the ruminal fermentation profile without affecting feed intake and milk production of mid-lactating dairy cows.

Key Words: aerobic stability, alcohol, heterofermentative inoculant, voluntary feed intake

Introduction

Whole crop corn is an easy forage to ensile and efficiently preserved under anaerobic conditions, whereas corn silage is highly prone to deterioration upon air exposure. The low aerobic stability is associated with high content of lactic acid and soluble sugars and low concentration of fermentation-end products with antifungal capability (Muck, 2010).

Heterofermentative bacteria, such as *Lactobacillus buchneri,* have been studied to improve the aerobic stability of corn silages, because they synthetize significant amounts of acetic acid (Kleinschmit and Kung, 2006). Besides acetic acid, the metabolism of lactic acid by *L. buchneri* also produces 1,2-propanediol (Oude Elferink et al., 2001), which can be further converted to 1-propanol and propionic acid by *Lactobacillus diolivorans* (Krooneman et al., 2002). Both acetic acid and propionic acid have antifungal action (Moon, 1983) and, therefore, can be effective to improve the aerobic stability of silages.

It is commonly observed that silages with heterofermentative fermentation patterns have more complex headspace-profiles than silages dominated by lactic acid fermentation (Kristensen et al., 2010), leading to an intense debate among researchers and field consultants on the possible negative effects of silages with high concentrations of volatile compounds (e.g., alcohols and esters) on the performance of dairy cows (Raun and Kristensen, 2010; Weiss et al., 2016). We have recently demonstrated that silages inoculated with high doses

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of *L. buchneri* depress the dry matter (DM) intake and milk yield of lactating dairy cows (Kleinshmitt et al., 2013). A headspace analysis by gas chromatograph-mass spectrometry indicated that 1-propanol was the major volatile compound in those silages inoculated with *L. buchneri* (unpublished data) and may be involved with the lower feed intake. A positive correlation between 1-propanol and heterofermentation has been previously reported (Raun and Kristensen, 2010).

Thus, the objective of this study was to investigate the effects of *L. buchneri* inoculation and 1-propanol supplementation on the nutritive value of corn silage for dairy cows.

Material and Methods

The corn crop (hybrid Agroceres AG 1051) was harvested at 350 g/kg of dry matter (DM), using a pull-type machine, without kernel processor, adjusted to a theoretical 8-mm length of cut. The chopped forage was packed in nine bag silos (1.5 m Ø × 14 m length; Pacifil, Estância Velha, Brazil), each with a capacity of 13 tons. The fresh forage contained (DM basis): 86.4 g/kg crude protein (CP), 519 g/kg of neutral detergent fiber (NDF), 34.7 g/kg ether extract (EE), 44.4 g/kg ash, 316 g/kg non-fiber carbohydrates (NFC), and 72 g/kg ethanol-soluble carbohydrates.

Two treatments were imposed at ensiling: control (no additive; six silos) and *L. buchneri* at 1×10^5 cfu/g (three silos). The microbial inoculant (*L. buchneri* CNCM I - 4323, Lallemand Animal Nutrition, Aparecida de Goiânia, Brazil) was diluted in distilled water (4 L/t) and sprayed onto the forage during packing. The same amount of water was added to the control silos.

Two control silos and one inoculated silo were opened in each experimental period of the lactation trial, starting after 247 days of ensiling. Silages were manually fed out from the silos, twice daily, immediately before feeding. Silages from the two control silos were homogenized and divided into two piles. One was used to compose the control diet and the second to compose the diet supplemented with 1-propanol. The number of control silos was the double of *L. buchneri* silos to keep the same feedout rate.

Silo working face (panel) was evaluated once in each sampling period of the lactation trial. After silage feedout in the morning, silage temperature was measured using bulb thermometers in five points distributed across the silo working face. The thermometers were positioned perpendicular to the silo panel at 10 cm deep.

Additionally, silage samples were collected for preparing aqueous extracts (1:10) using a stomacher homogenization for 4 min. The pH of the silage extract was evaluated using a digital pH meter (DM20, Digmed, São Paulo, Brazil). After filtering, the extract was centrifuged for 15 min at 10,000 × *g* and the supernatant was frozen at –20 ºC for later analysis of fermentation end-products. Analyses of acetone, esters, alcohols, and volatile fatty acids were performed using a gas chromatograph with a mass detector (GC-MS) (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, USA, 60 m, 0.25 mm Ø, 0.25 µm crossbond carbowax polyethlylene glycol) and analytical parameters according to the manufacturer's recommendation. Lactic acid concentration was determined by the colorimetric method proposed by Pryce (1969). The content of ammonia nitrogen (NH_3-N) was determined by colorimetry according to Chaney and Marbach (1962). The concentration of ethanol-soluble carbohydrates was analyzed in alcoholic extract by colorimetry using the phenol-sulfuric method (Hall, 2000).

Silage extracts were further diluted (10^{-2} to 10^{-6}) in Ringer's solution for microbial counts by pour plating in Petri dishes using selective media. Malt extract agar (Himedia, Mumbai, India) was used for enumeration of yeasts and molds after 48 and 72 h of incubation at 30 °C. Lactic acid bacteria (LAB) were enumerated using de Man, Rogosa, and Sharpe agar (Acumedia, Lansing, USA) after 48 h of incubation at 30 °C. Plaques with more than 20 and less than 300 colonies were selected and the number of colony forming units (cfu) was expressed in $\log_{10}$.

An aerobic stability test was performed in a room with controlled temperature (25±1 °C). Samples of 3 kg of each silage (control and *L. buchneri*) were loosely allocated in plastic buckets. A temperature sensor (Novus, Porto Alegre, Brazil) was placed at the geometric center of each bucket for recording the temperature every 30 min. The aerobic stability was defined as the time required for raising the silage temperature 2 °C above the ambient temperature (Moran et al., 1996). Additionally, temperature accumulation (°C) at five and ten days of exposure were computed as an indicator of aerobic deterioration (O'Kiely, 1993). For all silage variables, data from the two control silos were grouped and considered as an average value for each evaluation.

Twenty-one multiparous Holstein cows (BW = 663±100 kg) with an average of 230 days in milk at the beginning of the experiment, were randomly allocated in seven balanced 3 × 3 Latin squares, with 21-day periods (14 days of adaptation). Animals were housed in a tie-stall barn with a cooling system (micro sprays and fans) and individual feedbunks and water bowls. Cows were milked twice daily at 06:00 and 17:00 h. Animals were cared and

all procedures performed in compliance with accepted protocols (FASS, 2010).

Experimental diets contained (DM basis) 8 g/kg cottonseed, 95 g/kg citrus pulp, 180 g/kg soybean meal, 90 g/kg dry ground corn, 25 g/kg minerals + vitamins premix, and 530 g/kg of corn silage (control or inoculated with *L. buchneri* or control added with 1-propanol). The 1-propanol was dissolved in water (1:1) and sprinkled onto the total ration during mixing before each feeding to achieve a concentration of 10 g/kg of diet DM. Silages and concentrates were mixed by a self-propelled wagon (Data Ranger, American Calan, Northwood, USA) for 15 min. Diets were offered twice daily at 7:00 and 18:00 h and the amount of feed was adjusted daily to allow 100 g/kg of orts (as fed basis).

The voluntary dry matter intake (DMI) and milk production were recorded between day 15 and day 21 of each period. Milk samples were collected in four milking sections for analyses of protein, fat, lactose, total solids, and milk urea nitrogen (MUN) by infrared spectrometry (MilkoScan FT + 6000), and somatic cell count (SCC) by flow cytometry (Fossomatic 400; Clínica do Leite, Piracicaba, Brazil). Final concentrations of milk components were calculated after adjustment for milk production in each milking. Milk energy excretion (NE_L, Mcal/day) was estimated by multiplying milk yield (kg/d) by the milk energy content (Mcal/kg), calculated as 0.00929 × fat (g/kg) + 0.00547 × protein (g/kg) + 0.00395 × lactose (g/kg) (NRC, 2001).

Chewing behavior was recorded on day 15 by visual observation of the animals throughout a 24-h period to evaluate the possible negative effects of silage fermentation end-products on the eating patterns. Eating and ruminating activities were recorded at 10-min intervals and the 24-h pattern was estimated assuming a constant ingestive behavior between the observations (Maekawa et al., 2002). Chewing was computed as eating + ruminating. Samples of silages, total mixed rations, and orts were also collected to determine the particle size using the Penn State Particle Size Separator (Lammers et al., 1996). Sorting index was determined based on the observed intake of each particle size fraction expressed as a proportion of the predicted intake (g/kg as fed). Values <1000 g/kg indicate selective refusal, values >1000 g/kg indicate preferential intake, and values equal to 1000 g/kg indicate no sorting (Leonardi and Armentano, 2003).

Blood samples were obtained from coccygeal vessels on day 18 of each period, 2 h after the morning feeding. Blood was collected in vacuum tubes containing EDTA + sodium fluoride. Plasma was separated by centrifugation (2.000 × *g* for 20 min) and analyzed for glucose (enzymatic kit Glucose HK Liquiform, Labtest Diagnóstica S.A., Lagoa Santa, Brazil) and non-esterified fatty acids (NEFA) (kit Wako, NEFA C, kit no. 994-75409E, Richmond, USA).

Apparent total tract digestibility was determined using indigestible NDF (iNDF) as internal marker. Fecal samples were collected from day 16 to day 20 and composited by cow in each period. Samples of individual ingredients and dried feces were incubated for 288 h in the rumen of a cannulated cow receiving the control diet (Huhtanen et al., 1994). Cows were weighed on the first day of each period and at the end of the trial. Energy requirement for maintenance (NE_L maintenance, Mcal/day) was calculated in each period as 0.08 × average $BW^{0.75}$ (NRC, 2001).

Ruminal fluid was sampled through a flexible esophageal tube connected to a vacuum pump on the last day of the experiment (only in the last period), 2 h after the evening feeding. Samples of approximately 200 mL were used for measuring pH and frozen at –20 °C for analysis of acetate, propionate, butyrate, 1-propanol, and 1,2-propanediol by GC-MS, as described by silage fermentation end-products.

Samples of silages, concentrates, and orts were dried in a forced-ventilation oven at 55 °C for 72 h and ground through a 1-mm screen (Wiley mill). The concentrations of DM (method number 930.15), ash (method number 924.05), and EE (method number 920.39) were determined according to the AOAC (1990). The DM was corrected for volatile compounds according to Weissbach (2009). The concentration of CP was determined by combustion according to the method described by Wiles et al. (1998). The content of NDF was determined with an ANKOM® Fiber Analyzer (ANKOM Technology Corporation, Fairport, USA) using amylase and sodium sulfite (Mertens, 2002). The content of organic matter (OM) was calculated by discounting the ash content of the DM content. The NFC was calculated as 100 – ash – CP – NDF – EE (NRC, 2001).

Data of animal performance were analyzed using the Mixed procedure of SAS (Statistical Analysis System, version 9.3). The final model included the fixed effects of treatment and period and random effect of cow. The fixed effect of treatment × period was initially tested, but dropped from the model, since it was not significant. Autoregressive order 1 was used as the covariance structure and cow was the subject (Tempelman, 2004). Means were compared by the Tukey-Kramer test ($\alpha = 0.05$ and $\alpha = 0.10$). The concentrations of ruminal fermentation products were compared considering the random effect of Latin square and the fixed effect of treatment. The composition of the silages was analyzed using a model that included fixed effects of treatment and random effect of period.

Results

The LAB count was greater in *L. buchneri* silage, whereas the remaining variables were not affected by treatments, including the concentrations of fermentation products and aerobic stability (Table 1). Therefore, the compositions of the experimental diets were similar (Table 2).

No changes were observed in feeding behavior and DMI of cows fed silage inoculated with *L. buchneri* or supplemented with 1-propanol. In general, animals preferred shorter particles (<19 mm), but cows fed *L. buchneri* showed less preference for long particles (>19 mm) in favor of finer particles (<8 mm). Nonetheless, there were no differences in milk yield and milk composition across treatments (Tables 3 and 4).

Apparent digestibility of nutrients and energy partitioning were not affected by treatments, but diet NE_L was numerically lower for *L. buchneri*. Therefore, cows fed *L. buchneri* tended to have lower energy balance (Table 5). Proportions of major rumen volatile fatty acids were not modified by diets. In contrast, 1-propanol was increased in the rumen of cows fed the 1-propanol diet. Similarly, blood glucose concentration tended to be higher and NEFA concentration was lower for the 1-propanol treatment (Table 6).

Table 1 - Composition and management traits of experimental corn silages (g/kg DM, unless otherwise stated)

Item[1]	Treatment[2]		SEM	P-value
	Control	*L. buchneri*		
Dry matter (g/kg as fed)	353	355	0.8	0.63
Ethanol soluble carbohydrates	32.9	26.3	0.23	0.13
N-NH_3 (g/kg N)	84.4	75.4	0.32	0.14
pH	3.85	3.80	0.04	0.46
Lactic acid	32.7	33.3	0.52	0.91
Acetic acid	13.0	13.8	0.40	0.54
Propionic acid	8.87	8.99	0.23	0.86
1,2-propanediol	6.88	7.80	0.19	0.69
Butyric acid	3.91	3.99	0.14	0.86
Ethanol	3.50	4.99	0.09	0.34
2,3-Butanediol	1.24	0.92	0.10	0.34
1-propanol	0.67	1.01	0.03	0.38
Valeric acid (mg/kg DM)	652	657	128	0.93
i-Valeric acid (mg/kg DM)	491	492	85	0.98
i-Propyl alcohol (mg/kg DM)	36	49	15	0.65
Ethyl lactate (mg/kg DM)	23	38	10	0.21
Ethyl acetate (mg/kg DM)	12	32	7	0.14
2-Butanol (mg/kg DM)	5	11	4	0.14
Propyl acetate (mg/kg DM)	2	6	1	0.14
Lactic acid bacteria (log cfu/g as fed)	5.89	6.33	0.06	0.02
Yeast (log cfu/g as fed)	3.38	3.39	0.04	0.83
Mold (log cfu/g as fed)	2.74	2.35	0.39	0.55
Removal rate (m/day)	0.430	0.403	0.052	0.18
Density (kg as fed/m^3)	422	391	14.4	0.23
Silo face temperature (°C)	29.3	28.9	0.17	0.21
Aerobic stability (h)	15.8	24.5	3.35	0.12
Accumulated temperature (day 5; °C)	64.1	54.8	5.47	0.35
Accumulated temperature (day 10; °C)	113	106	3.3	0.21

SEM - standard error of the mean.
[1] Dry matter (DM) corrected for volatile compounds.
[2] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1 × 10^5 cfu/g.

Discussion

Lactobacillus buchneri is a heterolactic bacterium used to improve the aerobic stability of silages. However, there are still questions about the potentially adverse effects of fermentation products found in silages inoculated with it on feed intake, metabolism, and animal performance.

Table 2 - Chemical composition of the experimental diets consumed by the animals (g/kg dry matter, unless otherwise stated)

Item	Treatment[1]		
	Control	*L. buchneri*	1-propanol
DM (g/kg as fed)	433	432	438
CP	178	177	176
NDF	322	337	319
iNDF	141	147	140
EE	42.5	42.9	42.1
Ash	53.8	56.6	53.2
NFC	404	386	410

DM - dry matter corrected for volatile compounds; CP - crude protein; NDF - neutral detergent fiber; iNDF - indigestible NDF; EE - ether extract; NFC - non-fiber carbohydrates.
[1] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1 × 10^5 cfu/g; 1-propanol - supplemented with 1-propanol at 10 g/kg dry matter.

Table 3 - Performance of dairy cows fed diets with corn silage inoculated with *L. buchneri* or supplemented with 1-propanol

Item	Treatment[1]			SEM	P-value
	Control	*L. buchneri*	1-propanol		
DMI (kg/day)	20.9	20.5	20.0	0.50	0.26
Milk yield (kg/day)	32.1	31.3	31.3	1.45	0.35
FCM (kg/day)	32.0	31.3	31.4	1.55	0.74
Fat (g/kg)	35.3	35.1	36.0	0.10	0.72
Fat (kg)	1.12	1.10	1.11	0.06	0.86
Protein (g/kg)	33.8	33.3	33.9	0.11	0.92
Protein (kg)	1.08	1.03	1.06	0.05	0.65
Casein (g/kg)	27.0	25.5	26.0	0.10	0.60
Lactose (g/kg)	45.8	45.9	45.1	0.05	0.34
SCC (×1000/mL)	174	107	220	36.2	0.12
MUN (mg/dL)	11.5	12.2	12.2	0.50	0.43
FFA (mmol/10 L)	2.19	1.85	1.52	0.27	0.19
NE_L milk (Mcal/kg)	0.691	0.688	0.687	0.013	0.96

DMI - dry matter intake; FCM - fat corrected milk (35 g/kg of fat); SCC - somatic cell count; MUN - milk urea nitrogen; FFA - free fatty acids; NE_L milk - milk energy; SEM - standard error of the mean.
[1] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1 × 10^5 cfu/g; 1-propanol - supplemented with 1-propanol at 10 g/kg dry matter.

Table 4 - Feeding behavior of dairy cows fed diets with corn silage inoculated with *L. buchneri* or supplemented with 1-propanol

Item	Treatment[1]			SEM	P-value
	Control	*L. buchneri*	1-propanol		
Eating (min/day)	177	191	176	12.1	0.51
Ruminating (min/day)	527	522	507	14.9	0.45
Chewing (min/day)	704	711	679	20.5	0.32
Chewing/DM intake (min/kg)	34.8	35.7	33.9	1.55	0.39
Chewing/NDF intake (min/kg)	110	110	111	5.0	0.96
Sorting index					
>19 mm (g/kg as fed)	616a	445b	691a	35.0	<0.01
8-19 mm (g/kg as fed)	981	983	986	2.7	0.36
<8 mm (g/kg as fed)	1138xy	1157x	1118y	11.8	0.08

DM - dry matter corrected for volatiles; NDF - neutral detergent fiber; SEM - standard error of the mean.
[1] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1×10^5 cfu/g; 1-propanol - supplemented with 1-propanol at 10 g/kg dry matter.
Means followed by different letters within the same row differ from each other by the Tukey-Kramer test at $\alpha = 0.05$ (a,b) or $\alpha = 0.10$ (x,y).

Table 5 - Digestibility and energy partitioning in dairy cow diets based on corn silage inoculated with *L. buchneri* or supplemented with 1-propanol

Item	Treatment[1]			SEM	P-value
	Control	*L. buchneri*	1-propanol		
DM (g/kg)	688	676	689	6.7	0.23
CP (g/kg)	737	725	735	10.3	0.64
NDF (g/kg)	339	329	332	11.1	0.74
NFC (g/kg)	965	961	969	3.7	0.16
EE (g/kg)	924	915	924	4.1	0.13
TDN (g/kg)	728	717	723	5.7	0.37
NE_L diet (Mcal/kg)[2]	1.58	1.54	1.59	0.19	0.15
NE_L intake (Mcal/day)	32.5	30.9	31.5	0.89	0.19
NE_L maintenance (Mcal/day)	10.4	10.4	10.4	0.32	0.20
NE_L lactation (Mcal/day)	22.2	21.5	21.1	1.17	0.42
NE_L balance (Mcal/day)	2.98x	0.03y	1.19x	1.27	0.08
BW (kg)	661	663	659	26.6	0.14
BW change (kg/day)	0.325	0.168	0.259	0.086	0.41

DM - dry matter corrected for volatiles; CP - crude protein, NDF - neutral detergent fiber, NFC - non-fiber carbohydrates, EE - ether extract, TDN - total digestible nutrients, NE_L - net energy for lactation, BW - body weight.
[1] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1×10^5 cfu/g; 1-propanol - supplemented with 1-propanol at 10 g/kg dry matter.
[2] Calculated with digestibility data (NRC, 2001).
x,y - Means followed by different letters within the same row differ from each other by the Tukey-Kramer test at $\alpha = 0.10$.

Table 6 - Ruminal fermentation products (mol/100 mol) and blood metabolites of dairy cows fed diets with corn silage inoculated with *L. buchneri* or supplemented with 1-propanol

Item	Treatment[1]			SEM	P-value
	Control	*L. buchneri*	1-propanol		
Ruminal fermentation product					
Acetate	56.2	57.6	56.8	3.46	0.96
Propionate	25.5	25.1	25.0	1.52	0.97
Butyrate	16.4	18.1	16.0	1.36	0.55
1- Propanol	0.015b	0.005b	0.500a	0.111	0.04
1,2-Propanediol	0.163	0.113	0.140	0.027	0.48
Acetate:propionate ratio	2.27	2.40	2.26	0.32	0.93
Blood metabolite					
Glucose (mg/dL)	59.1y	59.1y	64.3x	1.93	0.08
NEFA (mmol/L)	0.184a	0.189a	0.157b	0.011	0.04

NEFA - non-esterified fatty acids; SEM - standard error of the mean.
[1] Control - no additive; *L. buchneri* - inoculated with *L. buchneri* at 1×10^5 cfu/g; 1-propanol - supplemented with 1-propanol at 10 g/kg dry matter.
Means followed by different letters within the same row differ from each other by the Tukey-Kramer test at $\alpha = 0.05$ (a,b) or $\alpha = 0.10$ (x,y).

As reported in the literature, one of the metabolic pathways of *L. buchneri* is the conversion of lactic acid to acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). In addition, 1,2-propanediol can be converted to 1-propanol and propionic acid by *Lactobacillus diolivorans* (Krooneman et al., 2002). Therefore, silages inoculated with *L. buchneri* may have high concentrations of acetic acid (Driehuis et al., 1999a; Ranjit and Kung, 2000; Hu et al., 2009), 1,2-propanodiol (Nishino et al., 2002; Driehuis et al., 1999b), propionic acid (Driehuis et al., 1999a; Wu-Tai et al., 2002), and 1-propanol (Driehuis et al., 1999a; Wu-Tai et al., 2002; Kristensen et al., 2010; Li and Nishino, 2011). In the current experiment, the dose of 1×10^5 cfu/g was not enough to raise the concentrations of these fermentation products in our silages.

Several studies reported improvements in aerobic stability of silage inoculated with *L. buchneri*, simultaneously to greater acetic acid concentration and lower yeast population (Ranjit and Kung, 2000; Filya, 2003; Schmidt and Kung, 2010). Therefore, the lack of benefit in aerobic stability for silages inoculated with *L. buchneri* in the current study might be mainly explained by the lack of response in acetic acid concentration and yeast counts. In Brazil, the typical commercial dose of inoculants containing *L. buchneri* is 1×10^5 cfu/g of forage. However, this low dose may be a plausible explanation for our results, because the changes in parameters such as aerobic stability and accumulated temperatures were mild regarding the control silage. In most studies in which benefits of aerobic stability were found with use of *L. buchneri*, the application rate was higher than the dose adopted in the present work (Nishino et al., 2004; Muck, 2004; Weinberg et al., 2002; Filya et al., 2006; Hu et al., 2009). In this way, Kleinschmit and Kung (2006) conducted a meta-analysis on the effects of *L. buchneri* on silages. The studies were stratified by crop and application rate in: control (untreated), 1×10^5 cfu/g, and $>1 \times 10^5$ cfu/g forage. For corn, silages were compiled over 26 experiments and in most studies, significant responses in aerobic stability were only obtained for inoculation doses greater than 1×10^5 cfu/g (Kleinschmit and Kung, 2006), which might support the absence of benefits of *L. buchneri* in the current trial.

Milk yield was not affected by treatments, probably due to a similarity in DMI and digestibility of nutrients. In the study conducted by Kristensen et al. (2007), there were also no changes in DMI and milk yield of primiparous cows fitted with permanent indwelling catheters in splanchnic tissues and receiving 1-propanol (5.6 g/kg of diet DM). Likewise, Raun and Kristensen (2012) supplemented a high dose of 1-propanol (50 g/kg of diet DM), which is not typical in silages, to challenge multiparous cows fitted with permanent indwelling catheters in splanchnic tissues. They did not observe changes in DMI and milk yield, but reported lower content of milk fat in cows supplemented with 1-propanol.

There are few studies reporting the effects of corn silage inoculation with *L. buchneri* on the performance of dairy cows. Kristensen et al. (2010) tested a dose of 3×10^5 cfu/g of *L. buchneri* in corn silage (comprising 390 g/kg of diet DM) in dairy farms and found no differences in the *in vitro* DM digestibility and milk yield (30.6 and 31.3 kg/d for control and inoculated silage, respectively). Driehuis et al. (1999b) used a dose of 1×10^5 cfu/g of *L. buchneri* in corn silage (comprising 330 g/kg of diet DM) for dairy cows, but did not observe differences in DMI, milk yield, or milk composition. In the present experiment, corn silage was the sole forage source and the inclusion in the diets (530 g/kg DM) was higher than those in the referenced studies. Notwithstanding, acetic acid concentration was low and similar across treatments (13.0 and 13.8 g/kg for the control and inoculated silages, respectively), with no interference on animal performance. Previous studies reported a clear negative effect of acetic acid on feeding behavior, but the dietary concentrations of acetic acid were higher than those found in the present trial (Dinius et al., 1968; Hutchinson and Wilkins, 1971; Krizsan et al., 2012; Daniel et al., 2013).

Despite similarities in NE_L utilization for maintenance and milk yield, cows fed silage inoculated with *L. buchneri* showed a lower NE_L balance. The numerical difference in BW variation suggests a lower accretion of body reserves in cows fed diet with *L. buchneri*. Blood glucose concentration was higher in cows fed diets supplemented with 1-propanol. Although the proportions of acetate and propionate in the rumen fluid were not modified across treatments, animals that received 1-propanol showed higher proportions of this alcohol in the rumen. Possibly, 1-propanol was partially recovered in the portal blood and converted to glucose in the liver (Czerkawski et al., 1984; Cozzi et al., 1996). Raun and Kristensen (2012) reported a portal recovery of 48 to 61% of the 1-propanol intake, with a consequent increase in hepatic uptake of 1-propanol, increased hepatic flow of glucose, and reduction in milk fat content. In the current trial, the improvement in the glucogenic status led to lower mobilization of triglycerides and lower blood concentration of NEFA in animals supplemented with 1-propanol. However, the increased glucogenic status induced by 1-propanol may not have been enough to depress the milk fat content in our mid-lactation cows.

Conclusions

Diets containing corn silages inoculated with *Lactobacillus buchneri* at 1×10^5 cfu/g or 10 g/kg of 1-propanol (dry matter basis) do not change the performance of mid-lactation dairy cows.

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