

# Effect of a homofermentative lactic acid bacteria additive on the quality of sugar-beet pulp silage and animal response of dairy cows with restricted access to pasture

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## ABSTRACT

Sugar-beet (*Beta vulgaris* L. subsp. *vulgaris*) pulp is a valuable by-product from the manufacture of beet sugar fresh which is normally preserved in the form of silage. The aim of the present research was to evaluate the effect of adding a homofermentative lactic acid bacteria silage additive during the ensiling period on fermentation and quality of sugar-beet (SB) pulp silage and the animal performance of dairy cows given restricted daily access to pasture. Twelve Holstein × Friesian crossbred cows were assigned to two groups according to milk yield, body weight, days in milk, parity and body condition score. Groups were randomly allocated to two treatments: silage of SB pulp without additive (control diet, SB) and silage of SB pulp with additive (SB+additive). The experiment was carried out in two periods of 28 d each. Once finished each period, cows were crossed for treatments. Chemical composition of SB silage was modified by the additive: Neutral detergent fiber (41.8% vs. 45.7% DM) and metabolizable energy (2.54 vs. 2.55 Mcal kg<sup>-1</sup> DM) were greater and butyrate lower (0.34 vs. 4.10 mmol L<sup>-1</sup>) for SB+additive compared with SB. However, milk production (24.6 and 25.2 kg d<sup>-1</sup>) and composition were not affected ( $P > 0.05$ ) by treatments. Similarly, rumen volatile fatty acids were similar between treatments. In conclusion, the use of homofermentative lactic acid additive on SB silage modified the chemical composition of silage and reduced the pasture intake, however, no changes in milk production and composition were detected.

**Key words:** *Beta vulgaris* subsp. *vulgaris*, milk production, rumen fermentation, silage additive.

## INTRODUCTION

In grazing-based dairy systems, variations in herbage growth rate and nutrient composition occur, causing herbage to be insufficient to meet the animal requirements during some periods such as winter and summer (Dillon, 2006). Restricting access time to pasture can be used as a management tool to increase grazing efficiency and pasture utilization (Chilibroste et al., 2007; Gregorini, 2012). Restricting herbage allowance or access time to pasture reduces herbage DM intake and milk production by dairy cows (Mattiauda et al., 2013), therefore it is necessary to provide supplementary feeds to meet the nutritional requirements of the milking herd (Peyraud and Delagarde, 2011). Supplementation with concentrates, silages, forage crops and/or by-products are common practices to improve nutritional status, and thereby milk yield in grazing dairy systems.

Feeding sugar-beet (*Beta vulgaris* L. subsp. *vulgaris*) (SB) pulp by-products is a viable option to improve sustainability of dairy production systems by decreasing the dietary proportion of human edible products (Ertl et al., 2015). Sugar-beet pulp is common ingredient in concentrate of dairy cows in many parts of the world, serving as an energy source, and offering several advantages over the traditional high energy grains that are used in dairy cattle feeding programs (Münnich et al., 2017).

Sugar-beet pulp is available as either a wet (pressed shreds) and/or ensiled as a storage method. Because drying is an energy- and cost-intensive procedure, ensiling of pressed pulp has become a popular method of preservation in Europe (Weber et al., 2003). Dolezal et al. (2005) reported that ensiling SB pulp with a low DM content (15%-18%), increases the risk of undesired fermentation associated with increased production of volatile fatty acids (VFA), especially acetic and butyric acids, and alcohol. Many experiments have been made to find the best way to ensile SB pulp reducing losses during fermentation process. However, information available in the literature is less abundant and therefore less conclusive when sugar-beet pulp silage is preserved in the form of plastic bag silos. For decades, producers have had a wide variety of silage additives available to assist in forage preservation (Muck et al., 2018). Addition of silage additives could restrict and reduce fermentation, reducing DM losses from fermentation (Borreani et al., 2018). In addition, their main functions are either increase nutritional value of silage or improve fermentation so that storage losses are reduced.

It has been shown that in grazing systems the type and quality of silage can affect pasture consumption and therefore milk production responses (Ruiz-Albarrán et al., 2016). To the best of our knowledge, there is scarce information about beet pulp silage supplementation in grazing dairy cows. Thus, the aim of the present research was to evaluate the effect of adding a homofermentative lactic acid bacteria (LAB) silage-additive during the ensiling period on fermentation and quality of sugar-beet pulp silage and the animal performance of dairy cows given restricted daily access to pasture.

## MATERIALS AND METHODS

### Experimental site and design

All procedures in this study agreed with the Animal Welfare Committee of Universidad Austral de Chile. This trial was carried out at the Austral Agricultural Research Station of the Universidad Austral de Chile (39°47' S, 73°14' W) over a period of 56 d (10 October to 5 December 2018) in a change-over experimental design, with 21 d of adaptation to the diets and a 7-d measurement period. Once finished each period, cows were crossed for treatments.

The experiment was carried out on 6 ha grass pasture (59.5% *Lolium perenne* L., 3.0% *Trifolium repens* L., 2.2% weed, and 35.3% dead material).

Twelve Holstein × Friesian crossbred cows were assigned to two groups according to milk yield ( $28.5 \pm 2.1$  kg), body weight (BW) ( $574.5 \pm 58.5$  kg), days in milk ( $212.3 \pm 33.4$  d), parity ( $4.8 \pm 1.6$ ) and body condition score ( $3.13 \pm 0.33$ ). The groups were randomly allocated to two treatments: Silage of sugar-beet (*Beta vulgaris* L. subsp. *vulgaris*) pulp without additive (control diet, SB-additive), and silage of sugar-beet pulp with additive (SB+additive).

All cows received as diet a permanent pasture, SB pulp silage and a commercial concentrate (Table 1). The treatments were strip-grazed in the same paddock, separated by an electric fence and the cows were offered herbage allowance (measured at ground level) of 21 kg DM cow<sup>-1</sup> d<sup>-1</sup>, which was allocated daily at 09:00 h, allowing them to graze for 8 h. Areas provided for grazing were adjusted daily on a pre-grazing herbage mass (HM) basis, which was estimated from 100 measurements collected with a rising plate meter, and the corresponding herbage allowance. The difference between pre-grazing and post-grazing HM for each grazed strip was assumed to be consumed by the cows and represented the pasture DM intake per hectare. In this study fresh SB pulp (pressed shreds) were ensiled in round bales. All cows were offered 6.25 kg DM cow<sup>-1</sup> d<sup>-1</sup> SB pulp silage (with or without additive) in feeding pens at 16:00 h, and 3.5 kg DM cow<sup>-1</sup> d<sup>-1</sup> concentrate together with silage in the cowshed. Water was always free access in the paddock and in the cowshed.

Sugar-beet silage was made from the same batch of sugar beets ('Crispina KWS', KWS SAAT SE, Einbeck, Germany). The silage additive contained colony-forming units of LAB: *Lactococcus lactis* SR354, *Pediococcus pentosaceus* P6, *Enterococcus faecium* M74, and *Lactobacillus plantarum* MiLab 393.

**Table 1. Chemical composition of herbage and supplements offered to dairy cows during the experiment.**

	Concentrate	Sd <sup>1</sup>	SB-additive	SB+additive	SEM <sup>2</sup>	P-value	Herbage	Sd
DM, %	88.3	0.58	22.7	23.0	0.68	0.81	17.9	2.89
Ash	7.31	0.33	6.81	6.60	0.22	0.46	8.81	1.33
CP, % DM	10.3	0.03	9.1	8.7	0.19	0.19	20.1	2.42
NDF, % DM	20.4	0.04	41.8b	45.7a	0.65	0.01	44.6	4.07
ADF, % DM	7.53	0.16	24.4	23.5	0.29	0.12	22.1	1.15
ME, Mcal kg <sup>-1</sup> DM	2.97	0.01	2.54b	2.55a	0.02	0.02	2.75	0.02
NFC, % DM	58.4	0.43	41.9	38.8	0.89	0.07	24.6	7.94
pH	-	-	4.10	4.19	0.07	0.46	-	-
NH <sub>3</sub> -N, %	-	-	3.79	3.53	0.21	0.44	-	-
Acetate, mmol L <sup>-1</sup>	-	-	1.89	1.92	0.27	0.94	-	-
Butyrate, mmol L <sup>-1</sup>	-	-	4.10a	0.34b	0.20	<0.01	-	-

SB: Sugar-beet; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ME: metabolizable energy; NFC: non-fibrous carbohydrates; NH<sub>3</sub>-N: ammonia-N.

Means within a row with different letters differ ( $P < 0.05$ ).

<sup>1</sup>Standard deviation.

<sup>2</sup>Standard error of the mean.

## Measurements

Pre- and post-grazing HM and herbage allowance were estimated using an electronic rising plate meter (RPM; Jenquip F150 Electronic Pasture Meter, Fielding, New Zealand). A total of 50 RPM measurements were taken pre- and post-grazing in each group grazing area, and HM DM estimated using a specific equation for spring pastures in southern Chile (HM kg DM ha<sup>-1</sup> = RPM × 100 + 400) (Canseco et al., 2007). Herbage samples were collected three times per week before grazing at 08:30 h from herbage strips of each treatments to determine its nutritive value. Samples from herbage were collected using the hand-plucking technique. The weights of concentrate and SB pulp silage offered and refused were recorded daily to determine individual feed intake. Concentrate and silages were sampled during 3 d during sample weeks of the experiment. Herbage, concentrate and silages samples were frozen at -20 °C and later freeze-dried and ground through 1 mm sieve (Willey Mill, 158 Arthur H, Thomas, Philadelphia, Pennsylvania, USA) and stored for chemical composition analysis. Samples were analyzed for DM, crude protein (CP), acid detergent fiber (ADF) and ash according to AOAC (1996). Neutral detergent fiber (NDF) content was determined by the method described by Van Soest et al. (1991). Metabolizable energy (ME) was estimated by regression using the digestible organic matter on DM basis (DOMD) and was determined in vitro (Tilley and Terry, 1963), according to Goering and Van Soest (1970). The pH and ammonia N (NH<sub>3</sub>-N) in silage samples were derived by the method of AOAC (1996).

The cows were milked twice daily, and milk yields were recorded at each milking throughout the experimental period by an electronic device (MPC580 DeLaval, Tumba, Sweden). During the last week of each period, bulked morning and evening samples were analyzed for protein, fat and urea using infrared spectroscopy (Milko-scan, System 4300, Foss Electric, Hillerod, Denmark). Body weight was recorded twice a day after each milking using an automated system while cows were walking out of the milking shed. In addition, body condition score was recorded on a five-point scale (Ferguson et al., 1994).

For ruminal concentration of volatile fatty acids (VFA), rumen fluid was harvested by stomach tube (Flora Rumen Scoop; Prof-Products, Guelph, Ontario, Canada) before (15:00 h) and 7 h (22:00 h) after feeding sugar-beet pulp silage on days 4 and 5 of week for each period. Samples were strained through 4 layers of cheesecloth. A 10 mL sample was drawn off, mixed with 0.2 mL 50% (w/v) sulfuric acid, and stored at -20 °C pending determination of VFA. Rumen fluid was allowed to thaw for 16 h to 4 °C, and then centrifuged at 10 000 × g for 10 min at 4 °C. Six milliliters of supernatant were drawn off and then centrifuged at 10 000 × g for 10 min at 4 °C. Thawed supernatant of rumen fluid samples were analyzed for VFA by gas chromatography as described by Tavendale et al. (2005).

## Statistical analysis

Effect of treatments on milk production and milk composition were analyzed using a linear-mixed model (LMM) in SAS (SAS Institute, Cary, North Carolina, USA). The model included the fixed effects of treatment, period and their interaction, and the random effect of cow. Effect of treatments on rumen fermentation parameters were analyzed using

a LMM in SAS software. The model included the fixed effects of treatment, period, time of day and the interactions between treatment and time of day. Model also included the random effect of cow.

Chemical composition of pasture was analyzed using the PROC MIXED in SAS. The model included the fixed effects of treatment, day of sampling and their interaction, and the random effect of plot. Comparison between treatments was carried out using the Tukey test. Results were considered significant at  $P < 0.05$  and tendency at  $P < 0.1$ .

## RESULTS AND DISCUSSION

Results of the chemical composition of the herbage, concentrate and silages eaten are presented in Table 1. Chemical composition was similar between silages ( $P > 0.05$ ) for DM and CP, although the silage with additive had a higher concentration of NDF and ME and are in accordance with values reported by Wyss et al. (2015) for SB pressed silages. The type of inoculant depends on the most common microbial problems affecting losses and quality. Where inhibiting clostridial activity is the primary issue, traditional homofermentative strains are the most beneficial, lowering pH more rapidly compared with an untreated silage (Borreani et al., 2018). In this study, only the lower butyrate content agrees with Kung and Muck (1997) in that the addition of facultative heterofermenters bacteria as additive usually results in lower pH, acetic acid, butyric acid, and ammonia-N but higher in lactic acid content and exhibit better DM recovery compared with untreated silages. The lower butyric acid concentration in inoculated silage is in accordance with the meta-analysis reported by Oliveira et al. (2017), who stated that reductions in butyrate in response to LAB inoculation is the most consistent and among the most important benefits. Results of pasture management and intake are presented in Table 2. Pre- and post-grazing HM were unaffected by treatment, averaging 2769 and 1659 kg DM ha<sup>-1</sup>, respectively. Pasture DM intake (DMI) was greater ( $P < 0.01$ ) for treatment SB-additive compared with treatment SB+additive (8.45 and 8.20 kg DM d<sup>-1</sup>, respectively), but without any explanation for effect, similar than reported by meta-analysis of Oliveira et al. (2017). In addition, there was a tendency ( $P = 0.08$ ) to have a greater total DMI in treatment SB-additive compared with treatment SB+additive (17.6 and 16.9 kg DM d<sup>-1</sup>, respectively). Silage and concentrate DMI per cow were unaffected by treatment, averaging 5.50 and 3.5 kg DM d<sup>-1</sup>, respectively.

Results of milk production, milk composition, milk solids and body weight are presented in Table 3. Animal trials have also revealed that facultative heterofermenters bacteria have not only enhanced silage fermentation but also have improved milk production, daily gain, or feed efficiency (Weinberg and Muck, 1996; Oliveira et al., 2017). Milk production tended ( $P = 0.06$ ) to be 2% greater (+0.6 kg d<sup>-1</sup>) for SB+additive than for SB-additive. A recent meta-analysis of 31 lactating dairy cattle studies indicated that inoculation with homofermentative or facultative heterofermentative LAB increased raw milk production (0.37 kg d<sup>-1</sup>;  $P < 0.01$ ), with only a trend for increased DMI and no effect on feed efficiency (Oliveira et al., 2017). It is known that improvements in animal performance from feeding inoculated silage are difficult to explain (Muck et al., 2018), therefore, in this study, the greater production could be explained by the better content of NDF, ME and rumen fermentation in treated silage group and probably by the inhibition of detrimental microbes and toxin production (Ellis et al., 2016), interaction of LAB with rumen microbes, and alteration of rumen fermentation (Weinberg et al., 2003). The greater NDF concentration of SB+additive is not necessarily related to poorer animal performance, as NDF of sugar-beet

**Table 2. Grazing management and pasture, supplement and total intake of cows receiving a restricted daily access to pasture and supplemented with sugar-beet pulp silage with (SB+additive) and without (SB-additive) additive.**

	Treatment		SEM	P value
	SB-additive	SB+additive		
Pasture mass, kg DM ha <sup>-1</sup>				
Pre-grazing	2728	2809	114	0.26
Post-grazing	1651	1666	43	0.77
Daily intake, kg DM cow <sup>-1</sup>				
Pasture intake	8.5a	8.2b	0.02	< 0.01
Sugar beet silage	5.7	5.3	0.26	0.23
Concentrate	3.5	3.5	-	-
Total	17.6	16.9	0.26	0.08

Means within a row with different letters differ ( $P < 0.05$ ).

SEM: Standard error of the mean.

pulp is very low in lignin (Michel et al., 1988) and therefore highly digestible in the rumen (Kara, 2019). Loucka (2012) observed that sugar beet pulp silages without additives have a more of alcoholic fermentation and with higher proteolysis, factors that can negatively affect milk production. However, alcohol production and non-protein N were not assessed in our study, the greater butyric acid concentration in SB-additive suggests a more alcoholic fermentation and greater proteolysis, as it is associated with clostridial activity (Pahlow et al., 2003). In addition, the clostridial fermentation affects the hygienic quality of silage by creating conditions that permit an increase in the numbers of pathogenic organisms and their toxins (Flythe and Russell, 2004), and it is well known that mycotoxins have a negative impact on milk production responses (Rodriguez, 2014). Protein and fat concentration in milk were not affected by treatments, averaging 3.38% and 3.86%, respectively. In addition, despite differences in the concentration of butyric acid between silages, it did not affect the milk solids, urea content in milk and bodyweight between treatments ( $P > 0.05$ ), as has happened in other studies.

The analysis of the effect of the additive on rumen total short chain fatty acids (SCFA, mmol L<sup>-1</sup>) and SCFA composition (acetate, propionate and butyrate) are presented in Table 4. There is little information on the effects of ensilaged pressed SB pulp contained in dairy cow's ration. There were no effects of treatments on total or composition of SCFA, despite the greater ME and NDF when the additive was incorporated in the silage. Hall et al. (2015) noted that ruminal SCFA concentrations might not satisfactorily reflect treatment effects on ruminal fermentation without knowledge of rumen liquid volume, which was not analyzed in our study. It was observed a tendency to greater propionate proportion for SB-additive after 6 h of started the silage supplementation ( $P = 0.05$ ), because of its numerical greater butyrate and acetate concentration at the same time, supporting the dilution in the propionate proportion.

A greater rumen SCFA -specifically butyrate concentration- has been associated with greater DMI (Seymour et al., 2005), however, in the current experiment the greater intake for SB-additive was not reflected in a greater SCFA rumen concentration. The lack of effect could be associated with the lower silage chemical composition compared with SB+additive (higher NDF and ME), suggesting that lower DMI was compensated by greater quality of nutrient intake, supporting the similar rumen fermentation parameters and milk production found in the current study.

**Table 3. Milk production, milk composition, milk solids and bodyweight of grazing dairy cows receiving a restricted daily access to pasture and supplemented with sugar-beet pulp silage with (SB+additive) and without (SB-additive) additive.**

	Treatments		SEM	P value
	SB-additive	SB+additive		
Milk production, kg d <sup>-1</sup>	24.6	25.2	0.60	0.06
Milk fat, %	3.84	3.87	0.16	0.87
Milk protein, %	3.42	3.33	0.08	0.22
Milk fat, g d <sup>-1</sup>	914.3	971.2	42.5	0.31
Milk protein, g d <sup>-1</sup>	834.1	831.9	18.3	0.92
Milk urea, mg L <sup>-1</sup>	190.8	174.7	21.0	0.60
Live weight, kg	592.0	592.0	12.6	0.98

SEM: Standard error of the mean.

**Table 4. Ruminal concentrations of acetate, butyrate, propionate and ammonia of grazing dairy cows receiving a restricted daily access to pasture and supplemented with sugar-beet pulp silage with (SB+additive) and without (SB-additive) additive.**

	Treatments			Time of day			P value		
	SB-additive	SB+additive	SEM	Before supplementation	After supplementation	SEM	Trt	Time	Trt × Time
SCFA, mmol L <sup>-1</sup>									
Acetate	42.27	43.25	3.82	47.98	41.54	3.82	0.59	0.27	0.83
Propionate	19.27	18.05	1.22	22.16a	15.16b	1.22	0.51	< 0.01	0.26
Butyrate	15.17	15.01	1.60	12.04b	18.14a	1.6	0.94	0.03	0.42
Total SCFA	76.24	80.71	5.88	88.28	68.67	5.88	0.61	0.05	0.73
SCFA, mol 100 mol <sup>-1</sup>									
Acetate	58.57	60.56	2.16	56.01	63.12	2.16	0.53	0.05	0.07
Propionate	22.54	21.00	1.13	23.82a	19.72b	1.13	0.37	0.03	0.05
Butyrate	18.88	18.42	1.42	20.16	17.14	1.42	0.83	0.7	0.24

Means within a row with different letters differ ( $P < 0.05$ ).

SCFA: Total short chain fatty acids; SEM: Standard error of the mean; Trt: treatment.

## CONCLUSIONS

Under the condition of the current experiment, the homofermentative lactic acid bacteria silage-additive added during the ensiling period of the beet sugar silage increased the neutral detergent fiber and metabolizable energy content of the silage and reduced its butyrate concentration. Additionally, the use of this additive reduced the DM intake, however, milk production tended to be greater in the cows who received the silage with additive.

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