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Chemical composition and in vitro rumen fermentation of ensiled sugar industry coproducts

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Abstract: The current study aims to compare sugar beet leaves (SBLs) ensiled with sugar beet pulp (SBP), sugarcane molasses (SM), or rock candy juice (RCJ) using an in vitro gas production technique. Treatments in the first group included 10% SBP (control 1) and control 1 plus 5% SM or RCJ. In the second group, there were 3 treatments, including 20% SBP (control 2) and control 2 plus 10% SM or RCJ. The silages with no added soluble carbohydrate (SC) sources had significantly higher ($P < 0.001$) pH, buffer value index, and ammonia nitrogen values than the other treatments. However, there was no significant difference between RCJ and SM in their effect on these variables. Adding both levels of SC sources caused an increase in dry matter digestibility compared to those that only included SBL and SBP ($P < 0.001$). Maximal and rate of gas production from slowly fermentable fraction (a_2) were significantly greater when the substrate contained 85%, 10%, and 5% SBL, SBP, and SC sources, respectively. Using dried SBP and SC sources for the preservation of fresh SBL is recommended; however, with higher levels of SC, the silage may be at risk of spoilage, and it must be less exposed to air.

Key word: Sugar beet leaves, soluble carbohydrates, sugar beet pulp, silage, rumen fermentation

1. Introduction

Twenty percent of the world's sugar production comes from sugar beets that are cultivated in many countries around the world, especially in temperate zones where sugarcane is not grown. A normal crop of sugar beets produces 40% to 50% of its weight as sugar beet tops, (1) which contain leaves and crown at a 60:40 ratio, respectively. The oxalic acid level in fresh sugar beet leaves (SBLs) can reach up to 9% of DM and the poisonous properties of the free acid and its soluble salts for sheep are widely recognized (2). However, silage oxalate is degraded to a considerable extent by the *Lactobacillus* species (3). Malavanh et al. (4) reported that ensiling the leaves of taro for 28 days with 4% molasses reduced oxalate concentration from 2.2% to 0.37% of DM. Therefore, ensiling SBL may improve the nutritive value of this byproduct.

The amount of soluble carbohydrates in beet leaves is lower than that of the protein fraction. Because of this and due to the high moisture content of sugar beet tops, it is very difficult to ensile this coproduct alone and ensilation with chemical and biological additives does not overcome the problem of nutrient loss through effluent production (5). Moreover, sun-drying of sugar beet tops is not feasible since the harvesting season is often characterized by low temperatures and cloudy weather. Therefore, in order to preserve SBLs as silage, both dry matter and carbohydrate content of this substance must be adjusted to optimum levels to get high-quality silage. Sugar beet pulp (SBP) is a valuable byproduct of the sugar beet industry and is widely used in dairy cow nutrition. SBP supplementation to the diet may have some effects on the degradation of forage fiber in the rumen due to the supply of readily fermentable pectin (6). Dried beet pulp is able to readily absorb water 4 to 5 times more than its weight. Hence, the addition of dried SBP to the leaves during the ensiling process may regulate the DM content of the leaves. Furthermore, it has been recommended that incorporation of absorbent products such as SBP into grass at ensiling time could improve animal performance and feed conversion efficiency compared to a situation where a similar quantity of the same absorbent product has been used with untreated silage (7).

Molasses is another byproduct of the sugar industry, and it is a potential energy supplement with high sugar content. This byproduct can supply enough energy for silage microbes, which consequently increase fermentation and thus enhance the production of lactic acid in the silage (8). Rock candy juice (RCJ) is a byproduct of the rock candy industry. It has similar chemical and physical properties to molasses, which is widely produced in countries (e.g., Iran) in which rock candy is regularly produced.

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Using SBL, SBP, and molasses as feed for ruminants has already been explored. However, there are no comprehensive data on the effect of a mixture of these byproducts in silage. It is widely believed that the replacement of conventional ingredients by a combination of alternative sources of protein, structural carbohydrates, and nonstructural carbohydrates could promote different ruminal fermentation (9). Accordingly, the present study was designed to investigate the effects of adding SBP and sugarcane molasses (SM) or RCJ to the SBL silage on the chemical composition, silage quality, and in vitro rumen fermentation of the experimental silages.

2. Materials and methods

2.1. Experimental silages

SBLs were collected from a farm near Mashhad, located in East Iran, and chopped to a length of 4 to 5 cm before ensiling. Within 2 to 3 h of chopping, the following groups of treatments were applied (fresh weight basis) to 3 kg of SBLs in triplicate: group 1 involved 10% SBP (control 1), control 1 plus 5% SM, and control 1 plus 5% RCJ. The second group included three treatments: 20% SBP (control 2), control 2 plus 10% SM, and control 2 plus 10% RCJ. At first, the researchers attempted to ensile SBL without adding SBP. However, within a few days, large amounts of stinking leachate appeared in the plastic bags. Therefore, the contents of these bags were not used for further analysis. SBP and SM were taken from the Dairy Research Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. In addition, RCJ was obtained from one of the rock candy producers near Mashhad.

All of the components of each treatment were properly hand-mixed, pressed into two layers of plastic bags in triplicate, packed, and then stored at room temperature (20 to 25 °C) for 60 days. The weights of green forage at the time of ensiling were recorded for the calculation of DM recovery. The plastic bags were opened after 2 months and 2 subsamples were taken for further usage. One sample was dried in an oven at 60 °C for 48 h and grounded with a laboratory mill (standard model 4, Arthur H. Thomas Co., Philadelphia, PA, USA), fitted with a 1-mm screen, and stored in two-layer polythene bags for gas production tests and chemical analysis. In order to measure the pH of the silage juice, 50 g of fresh silage was mixed with 450 mL of distilled water and homogenized for 3 min in a high-speed blender (10). Silage extracts were then filtered through 4 layers of cheesecloth and pH was instantly measured using a pH meter (Metrohm 691). Five milliliters of water extracted from the silage juice was used for measuring ammonia-N (8) according to the method described later in the relevant paper. Twenty milliliters of silage juice was kept at –20 °C to estimate lactic acid content using the calorimetric method (11) and total volatile fatty acid content was measured by steam distillation (12). Another 5 g of wet silage (equivalent to 0.5 g of DM) was also mixed with 30 mL of distilled water, homogenized for 3 m in a high-speed blender, and filtered through 4 layers of cheesecloth. Initial pH was recorded using a pH meter (Metrohm 691) after allowing 2 min for equilibrium. Also, buffering capacity (BC) was determined by titration against a 1 N NaOH solution. A subsample of about 100 g was exposed to air and covered with cheesecloth. Values of the temperature of the silages were then measured at the opening of the silages and subsequently every 24 h until day 5 to assess the heat increment of the silage. The temperature was measured using a digital thermometer with a penetration probe (Gulterm 180, Gulton, São Paulo, Brazil) positioned in the geometric center of the experimental silages. The ambient temperature was taken as a reference and was recorded at the same time as the pH and temperature measurements.

2.2. In vitro gas production

In vitro gas production was carried out in 2 separate runs with 3 replicates per substrate according to the method proposed by Menke and Steingass (13). Rumen content was obtained 2 h before the morning feeding from 2 ruminally cannulated Holstein steers given a diet containing alfalfa hay and concentrated mixture at a 60:40 ratio at a 2.5% body-weight level. The rumen content was collected in 2 insulated thermo flasks and immediately transferred to the laboratory. The content was filtered through 4 layers of cheesecloth to remove any contaminating particles that may have interfered with the dispensation of rumen fluid into the serum bottles and then kept at 39 °C in a water bath with continuous flushing of CO_2 to provide an oxygen-free environment. An anaerobic medium was prepared on the morning of incubation and warmed at 39 °C in a water bath. Particle-free ruminal fluid was anaerobically mixed with the buffer solution at a proportion of 1:2 (v/v) at 39 °C. Two hundred milligrams (DM basis, ±0.02) of each oven-dried silage was accurately weighed and transferred to a glass bottle (120 mL). Thirty milliliters of anaerobic buffered rumen content was anaerobically dispensed to prewarmed oxygen-free serum bottles, purged with CO_2 to remove air from the headspace, and sealed by rubber stopper and aluminum cup to prevent the escape of fermentation gases. Incubation was performed in a water bath at 39 °C for 48 h. Four additional substrate-free bottles served as blanks and 4 bottles containing standard hay (purchased from the University of Hohenheim) were considered to correct the difference among runs. A fully automated computerized gas measurement system (designed and developed at Ferdowsi University of Mashhad, Iran) was used for measuring the gas pressure produced from the fermentation of substrates at 2-min intervals. After 48 h of incubation, fermentation was arrested by chilling bottles to 4 °C, and the quantities of fermentation products were determined in each bottle using methods of analysis that will be described later. The contents of the bottles were transferred to a centrifuge tube and then centrifuged at 3000 rpm for 15 min. The pellets were transferred to spoutless beakers (500 mL) by dissolving them with 100 mL of neutral detergent solution. The beakers were kept on a heater and refluxed at 100 °C for 1 h from the time the boiling started. The contents of the beakers were filtered under vacuum through preweighed sintered crucibles with pore size p_2 (Foss, Hillerød, Denmark) and washed with hot water. The crucibles containing residue were oven-dried (65 °C for 48 h) and weighed, and the dried residue was ashed at 550 °C.

2.3. Laboratory analysis and calculation

All samples were assayed in triplicate for DM and crude protein (CP) according to AOAC methods 934.01 and 990.03, respectively (14); OM was determined by ashing at 550 °C for 5 h. Neutral detergent fiber was measured using the procedure proposed by Van Soest et al. (15). The following equations were used for calculating in vitro dry matter digestibility (IVDMD) and true organic matter digestibility (TOMD), as described by Ebrahimi et al. (16):

IVDMD (%) = ((S – (R – B)) / S) ×100,

where S is the weight of substrate (mg), R is the weight of dried residue after treatment with neutral detergent solution (mg), and B is the weight of the dried residue of representative blanks after treatment with neutral detergent solution (mg). Also:

TOMD (%) = ((OMS – (OMR – OMB))/OMS) × 100,

where OMS is the weight of OM in substrate (mg), OMR is the weight of OM residue in the crucible (mg), and OMB is the weight of OM residue in representative blanks (mg).

Partitioning factor (PF) and microbial biomass production (MBP) were calculated based on truly degraded organic matter (TDOM) using the following equations, respectively:

PF (mg/mL) = TDOM (mg)/net gas production,

where TDOM was calculated by multiplying TOMD [%] by ppm OM content of substrate, and:

MBP (mg) = TDOM (mg) – (2.25 × mL net gas volume),

where the constant 2.25 is the stoichiometric factor.

A two-phase model was used for the interpretation of gas production profile and the amount of gas was expressed as mL/g OM:

$$\text{mL gas} = \frac{a1}{(1+\left(\frac{b1}{t}\right)^{c1})} + \frac{a2}{(1+\left(\frac{b2}{t}\right)^{c2})}$$

where a_1 and a_2 are maximal gas production in mL from the rapidly fermentable fraction and slowly fermentable fraction, respectively; b_1 and b_2 are the time in which half of the maximal gas production (a_1, a_2) occurred in h, c is a parameter determining the shape of the curve; and t is time in h. The gas production curves were fitted by GraphPad Prism 6.

Ammonia-N was measured through the distillation method (Kjeltec Auto 1030 Analyzer Tecator, Hoganas, Sweden) according to the standard method (14). The BC of silages was calculated as milliequivalents per liter as follows:

BC = ((mL of 1 N NaOH × 10^3)/30).

The buffer value index (BVI), which positively correlates with BC but has a negative correlation with acidity, was determined as follows:

BVI = ((((antilog_{10} (–STPH)) – (antilog_{10} (–SAPH)))/(antilog_{10} (–STPH)) + (SABC – STBC)/STBC)) × 10) + 100,

where STPH is a standard pH of 6, SAPH is the sample pH, SABC is the sample BC (milliequivalents per liter), and STBC is a standard BC of 50 meq L^{-1}.

The Fleight point (FP) of the experimental silage was calculated by the following equation:

FP = 220 + (2 × % DM – 15) – (40 × pH).

An FP value of 85–100 represents a very good quality of silage, 60–80 good quality, 55–60 moderate quality, 25–40 satisfying quality, and <20 worthless.

Dry matter recovery was estimated according to the following equation:

DMR (%) = [(GMfo × DMfo)/(DMs × DMsi)] × 100,

in which DMR (%) = dry matter recovery; GMfo = green mass of the forage (kg) at the time of ensilage; DMfo = dry matter of forage (%) at the time of ensilage; DMs = dry matter of the silage (kg) at silage opening; and DMsi = dry matter of the silage (%) at silage opening.

2.4. Statistical analysis

Statistical analysis was conducted using the ANOVA procedure in SAS (SAS Institute Inc., USA). A completely randomized design was used for the analysis of treatments within each group with regards to silage parameters with the following model:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

where Y_{ij} is analytical data, μ is overall mean, T_i is the effect of the treatment, and ε_{ij} is the random error. $P < 0.01$ was considered to be statistically significant. Duncan's test was used to examine the significance degree among means.

3. Results

The chemical composition of the silages' components is presented in Table 1. Compared to the other constituents, SBL had a considerable amount of CP and ash content. Table 2 illustrates the proximate analysis of the experimental silages. As can be seen in the table, there were no significant differences between the DM level in silages containing RCJ and SM in the 2 groups of treatments. However, the existence of both soluble carbohydrates improved the

Table 1. Chemical composition of the components used for the experimental silages.

Item	SBL	SBP	RCJ	SM
DM (%)	12.05	97.13	72.80	70.58
OM (% of DM)	73.06	92.10	99.86	95.58
Ash (% of DM)	26.94	7.90	0.14	4.42
CP (% of DM)	19.82	14.3	1.60	7.29
NDF (% of DM)	41.55	52.40	0.00	0.00

SBL = Sugar beet leaves; SBP = sugar beet pulp; RCJ = rock candy juice; SM = sugarcane molasses; DM = dry matter; OM = organic matter; CP = crude protein, NDF = neutral detergent fiber.

DM content of the silage ($P < 0.001$). The presence of SM and RCJ led to a decrease in the NDF content of silages compared to the situation with no added water-soluble carbohydrates in both levels of SBP ($P < 0.001$). Trends were found indicating an increase in the CP content of the silages by adding SM or RCJ in two levels of SBP.

The fermentation parameters of the experimental silages are presented in Table 3. The silages with no added SC sources had significantly higher ($P < 0.001$) pH and BVI values than the other treatments. However, there was no significant difference between RCJ and SM in terms of their effect on the reduction of silage pH. In addition, inclusion of SM and RCJ in the SBL silage significantly reduced the N-NH_3 concentration ($P < 0.01$). In the silages with no added SC sources, lower values of BC were observed compared to silages including SC in both SBP levels ($P < 0.001$), although there was no significant difference between SM and RCJ in this regard. Apart from the level of SBP, the use of SM or RCJ for treating SBL silage led to a significant increase in dry matter recovery of the experimental silages ($P < 0.001$). Total volatile fatty acid and lactic acid was significantly higher in silages containing 5% or 10% RCJ or SM ($P < 0.0001$).

Figure 1 shows the changes of the pH in the silage samples exposed to air during the 120 h after opening them. Overall, in the silages with SC, pH values were below those without SC throughout the measuring period. However, in all of the treatments, an increase in pH value was observed up until the first 72 h. Temperature changes in the silages exposed to air are represented in Figure 2. It was found that for all treatments, the temperature of ensiled SB increased markedly up until 24 h of exposure to air, such that silages containing 10% SM had a higher temperature than others ($P < 0.01$) at 24 h after exposure to air. From this point on, all silages showed similar changes in temperature during the first 120 h of exposure to air.

Total gas production, digestibility, PF, and MBP of the incubated substrates are summarized in Table 4. For both groups, total gas production was greater in the substrates containing SC ($P < 0.05$). Adding 5% or 10% of soluble carbohydrate sources caused an increase in the digestibility compared to those that only included SBP and SBL ($P < 0.001$). The addition of SC did not have a significant effect on PF, but a significantly higher MBP was observed in these treatments as compared to silages without SM or RCJ.

The parameters of the gas production profile are presented in Table 5. For the group with 10% SBP, silages treated with SC had lower maximal gas production from rapid fermentation fraction than those without SC. In the second group, no significant effect was found in this regard. The rate of gas production from rapidly fermentable fraction in the silages containing SC was greater than those without SC. However, this effect was more considerable in silages having 10% SBP. Maximal and rate of gas production from slowly fermentable fraction (a_2) when the substrate contained 85%, 10%, and 5% SBL,

Table 2. Chemical composition of the experimental silages.

Item	10% SBP			SEM	P	20% SBP			SEM	P
	SM (5)	RCJ (5)	No SC			SM (10)	RCJ (10)	No SC		
DM (%)	18.66^a	18.51^a	15.68^b	0.21	0.003	27.44^a	27.49^a	23.33^b	0.21	<0.001
OM (% of DM)	80.63	80.68	78.01	0.42	0.06	85.40^a	85.39^a	81.77^b	0.29	0.003
CP (% of DM)	15.52	14.67	16.93	0.3	0.06	14.32^a	13.20^a	15.98^b	0.32	0.03
NDF (% of DM)	27.67^a	27.07^a	35.22^b	0.33	<0.001	35.58^a	35.40^a	41.57^b	0.45	0.0001

SBP = Sugar beet pulp; RCJ (5, 10) = rock candy juice at the level of 5% and 10% of fresh weight, respectively; SM (5, 10) = sugarcane molasses at the level of 5% and 10% of fresh weight, respectively; SC = soluble carbohydrate; DM = dry matter; OM = organic matter; CP = crude protein, NDF = neutral detergent fiber.
Means in rows without common superscripts are significantly different; SEM = standard error of the mean.

Table 3. Effect of treating SBL with SBP and SM or RCJ on fermentation parameters.

Item	10% SBP			SEM	P	20% SBP			SEM	P
	SM (5)	RCJ (5)	No SC			SM (10)	RCJ (10)	No SC		
pH	4.23[a]	4.15[a]	4.93[b]	0.03	<0.0001	4.15[b]	4.10[a]	4.82[b]	0.04	0.0003
$N\text{-}NH_3$ (mg/dL)	5.11[a]	5.48[a]	7.43[b]	0.12	0.0005	5.02	5.20	6.88	0.26	0.05
BC (meq L^{-1})	425.56[a]	438.89[a]	332.22[b]	5.48	0.0004	386.67[a]	403.33[a]	342.33[b]	4.64	0.004
BVI	−619.16[a]	−758.89[a]	−21.02[b]	68.70	0.01	−836.60[a]	−889.60[a]	−90.82[b]	42.49	0.0004
FP	73.12[b]	76.02[b]	39.16[d]	1.48	<0.0001	93.88[a]	95.98[a]	58.86[b]	1.44	<0.0001
DMR	74.84[a]	73.57[a]	59.25[b]	1.40	0.0006	81.77[a]	81.40[a]	65.49[b]	0.73	<0.0001
Lactic acid (DM %)	5.21[a]	5.18[a]	2.33[b]	0.16	<0.0001	5.71[a]	5.70[a]	3.60[b]	0.19	<0.0001
Total VFA (mmol/dL)	2.56[a]	2.59[a]	1.24[b]	0.01	<0.0001	2.63[a]	2.64[a]	1.42[b]	0.01	<0.0001

SBP = Sugar beet pulp; RCJ (5, 10) = rock candy juice at the level of 5% and 10% of fresh weight, respectively; SM (5, 10) = sugarcane molasses at the level of 5% and 10% of fresh weight, respectively; SC = soluble carbohydrate; BC = buffering capacity; BVI = buffer value index; FP = Fleight point; DMR = dry matter recovery.
Means in rows without common superscripts are significantly different; SEM = standard error of the mean.

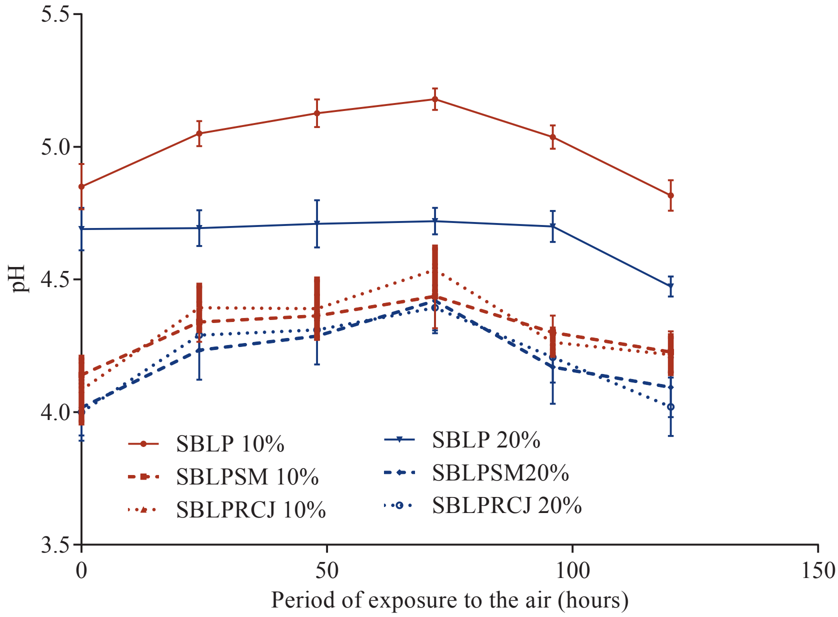

Figure 1. Mean ± standard error of pH in the experimental silages exposed to air at 0, 24, 48, 72, 96, and 120 h after opening the silages. SBLP 10% and 20%: Sugar beet leaves (SBL) with 10% and 20% added sugar beet pulp (SBP); SBLPRCJ and SBLPSM 10%: SBL with 5% rock candy juice (RCJ) or sugarcane molasses and 10% added SBP; SBLPRCJ and SBLPSM 20%: SBL with 10% rock candy juice (RCJ) or sugarcane molasses and 20% added SBP.

SBP, and SC, respectively, were significantly greater than other treatments but increasing the proportion of SBP to 20% reduced the extent and rate of the slowly fermentable fraction even with the existence of 10% SC.

4. Discussion

As previously mentioned, SBL contained remarkable ash content (26.94%, Table 1), which can be attributed to soil contamination during leaf harvesting as reported by

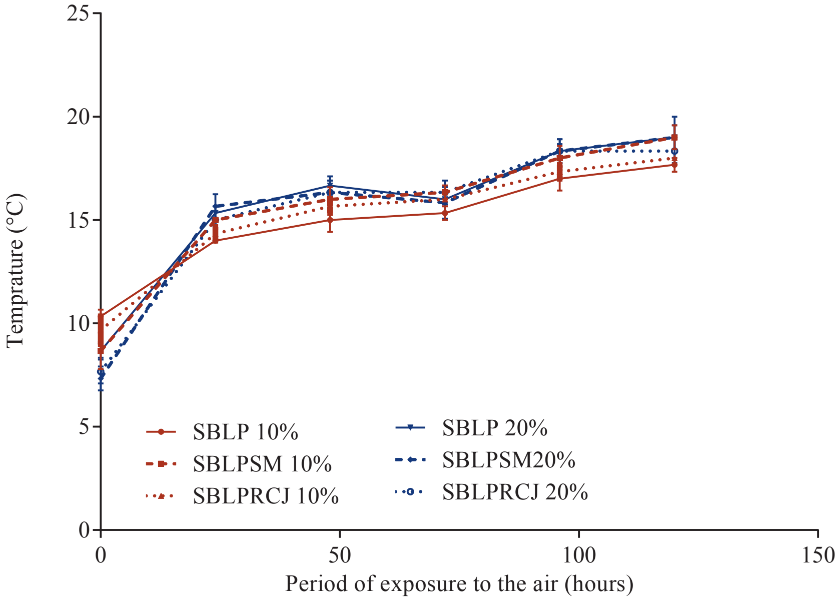

Figure 2. Changes in the temperature of experimental silages exposed to air at 0, 24, 48, 72, 96, and 120 h after opening the silages expressed as mean ± standard error. SBLP 10% and 20%: Sugar beet leaves (SBL) with 10% and 20% added sugar beet pulp (SBP); SBLPRCJ and SBLPSM 10%: SBL with 5% rock candy juice (RCJ) or sugarcane molasses and 10% added SBP; SBLPRCJ and SBLPSM 20%: SBL with 10% rock candy juice (RCJ) or sugarcane molasses and 20% added SBP.

Table 4. Effects of treating SBL with SBP and SM or RCJ on in vitro fermentation parameters of the incubated silages.

Item	10% SBP			SEM	P	20% SBP			SEM	P
	SM (5)	RCJ (5)	No SC			SM (10)	RCJ (10)	No SC		
Total gas (mL/g OM/48 h)	342.43^{ab}	395.13^{a}	329.76^{b}	11.73	0.01	356.04^{a}	337.87^{ab}	309.09^{b}	8.23	0.02
IVDMD (%)	85.15^{a}	84.89^{a}	79.07^{b}	0.29	<0.0001	86.05^{a}	86.10^{a}	80.66^{b}	0.39	<0.0001
TOMD (%)	84.97^{a}	86.78^{a}	81.14^{b}	0.55	<0.0001	89.75^{a}	90.41^{a}	82.26^{b}	0.69	0.001
PF (mg TDOM/mL net gas)	2.80	2.71	2.69	0.03	0.16	2.69	2.68	2.65	0.01	0.2
MBP (mg)	29.30^{a}	27.68^{a}	24.52^{b}	0.43	<0.0001	26.92^{bc}	25.29^{cd}	21.73^{e}	0.33	<0.0001

SBP = Sugar beet pulp; RCJ (5, 10) = rock candy juice at the level of 5% and 10% of fresh weight, respectively; SM (5, 10) = sugarcane molasses at the level of 5% and 10% of fresh weight, respectively; SC = soluble carbohydrate; IVDMD = in vitro dry matter digestibility; TOMD = true organic matter digestibility; TDON = truly degraded organic matter; PF = partitioning factor; MBP = microbial biomass production.
Means in rows without common superscripts are significantly different; SEM = standard error of mean.

Gurbuz and Kaplan (17). The crude protein content of sugar beet tops depends on the proportion of the leaves and crowns, and the CP content of leaves in the present study was within the range of values (17.7% to 24.5% DM) reported by Feedipedia (18) and Gurbuz and Kaplan (17). Dried SBP is known for its water absorption capability (19) and using this byproduct in combination with other high-moisture components while making silage had been previously practiced for modulating DM (5,20). Based on the data presented in Table 2, it seems that 20% SBP reduced the DM of the silages to about 27%, a value that is acceptable for making good-quality silages (21). This DM adjustment was accompanied by a decrease in the ash and CP contents but caused an increase in the NDF content of composite silages.

The parameters determining the quality of the silages are given in Table 3. Crops with high protein content (e.g., alfalfa) have a buffering capacity to resist pH decline

Table 5. Gas production parameters of incubated substrates.

Item	10% SBP			SEM	P	20% SBP			SEM	P
	SM (5)	RCJ (5)	No SC			SM (10)	RCJ (10)	No SC		
a_1	139.82[a]	169.03[ab]	189.80[b]	9.22	0.02	185.64	200.00	163.89	9.25	0.1
b_1	0.82[a]	0.89[a]	2.07[b]	0.1	<0.0001	1.24	2.09	1.78	0.24	0.16
c_1	0.76[a]	1.07[b]	0.98[b]	0.03	0.0003	1.05	0.78	1.05	0.08	0.09
a_2	214.33[a]	234.73[a]	111.62[b]	14.22	0.0003	174.68	154.78	156.22	13.04	0.62
b_2	6.32	6.36	7.03	0.28	0.29	5.58[a]	5.22[a]	6.73[b]	0.15	<0.0001
c_2	1.82[a]	1.96[a]	3.59[b]	0.07	<0.0001	2.74	2.69	2.69	0.19	0.98

SBP = Sugar beet pulp; RCJ (5, 10) = rock candy juice at the level of 5% and 10% of fresh weight, respectively; SM (5, 10) = sugarcane molasses at the level of 5% and 10% of fresh weight, respectively; SC= soluble carbohydrate.
Means in rows without common superscripts are significantly different; SEM = standard error of mean.
a_1 and a_2 = asymptotic maximal gas production (mL gas g^{-1} OM) from rapid and slowly fermentable fraction, respectively; b_1 and b_2 = the times (h) at which half of the maximum gas production (a_1 and a_2) are produced; c_1 and c_2 = parameters determining the shape of the curves.

during the ensiling process (22) and, as shown in Table 1, the CP value in the leaves of the sugar beet was high enough for this to take place. Another factor contributing to the decrease of pH in the silage of forage is the DM content (23). In the case of SBL, the DM content was quite low (Table 1). It appears from the pH values that using SBP and RCJ or SM together with SBL overcomes factors that could prevent the decrease of pH in the silage, and the presence of soluble carbohydrates was mandatory since silages without SC showed greater pH than those containing it. In other words, besides modulating the DM of the forage, there was a need for sources of soluble sugars (RCJ or SM) to stimulate silage fermentation towards lactic acid production and to lower the pH. Such a harmonious effect was found when SBL was ensiled with the maize crop (17) or molasses was added to the silages (11,24). Since silages containing RCJ and SM had lower pH levels and greater BC than those that included SBL and pulp, it could be concluded that SBL ensiling was more successful when both SBP and SC sources were added to SBL. To better understand the positive effect of RCJ and SM on the ensilation of SBL in the present study, the BC of the silages was expressed in terms of BVI (Table 3). The significant difference between the BVI values of silages with and without SC sources indicated that the main fermentation product of the silages containing RCJ and SM was lactic acid rather than volatile fatty acids such as acetic acid or butyric acid (22). When comparing the N-NH_3 of the silages, an increase in ammonia-N was observed in the absence of RCJ and SM, which implies that the fermentation of soluble carbohydrates resulted in an inhibition of proteolytic activity in the silages containing SC sources. This result is in line with the findings of Yuan et al. (25). Losses of DM in the silages were reduced following an increase in the level of SBP, which is consistent with the results obtained by Moore and Kennedy (7). Homofermentative lactic acid production only produces lactic acid and reduces carbon losses, which results in more DMR (23). Significantly higher FP and DMR in the silages with 10% and 20% SC sources and SBP confirmed this combination as the best quality silage among treatments, which is in accordance with the results reported by Gurbuz and Kaplan (17), who found an improvement in the SBL silage quality by coensiling it with corn forage.

As shown in Table 4, silages containing SM and RCJ produced higher gas after 48 h of incubation compared to those without SC sources. Part of the added SC was probably not fermented throughout silage fermentation and utilized by rumen microorganism during in vitro fermentation. However, since the increase of SC level was followed by a doubling of the SBP level, silages with 10% SC sources did not produce significantly greater gas than 5% RCJ or SM. The presence or absence of RCJ or SM was a major factor influencing in vitro DM digestibility as IVDMD and TOMD were significantly higher in silages containing SC sources (Table 4). Such improvement in digestibility possibly caused relatively more MBP in silages treated with RCJ and SM. Increased IVDMD and TOMD in silages following an increase in the level of SM and RCJ confirm the results of other studies that used molasses or sugar supplements (26,27). In the existing literature, Harland (28) reported a DM digestibility of 50% to 60%, and Gurbuz and Kaplan (17) found a low in vitro organic matter digestibility (36%) for ensiled SBL alone, values that are all less than the estimated values for SBL ensiled with SBP and SC sources in the current study. Presumably, coensiling SBL with two other components improved in vitro DM digestibility of SBL and/or a higher digestibility of SC and SBP led to greater values.

The amount of gas production per time unit or the rate of gas production can be calculated based on the values of maximal gas production and parameter *b*. In silages with 70%, 20%, and 10% of SBL, SBP, and SC sources, the substrate was fermented more slowly than in compositions with 85%, 10%, and 5% of SBL, SBP, and SC sources, respectively. This is probably due to the higher proportion of NDF provided by SBP in the composite silage.

Gomes et al. (29) noted that lactic acid degradation increased the pH of silages and caused the growth of many other spoilage organisms when silages were exposed to air. On the other hand, and as explained earlier, some of the added SC was not fermented and was used for further rumen fermentation instead. This could provide substrate for aerobic microorganisms, resulting in an increase in silage temperature and therefore lower aerobic stability for silages with SC sources. In other words, greater heat production is expected in silage containing higher DM (30). For the above reasons, while silages without SM or RCJ showed higher pH throughout the period of exposure to air, the inside temperature of those containing SC sources (particularly with high levels of additives) was relatively higher than silages only containing SBL and SBP (Figures 1 and 2).

Overall, the results of the present study have indicated that the addition of both SBP and RCJ or SM is necessary for obtaining good-quality silages. There was no difference between RCJ and SM in this regard. When RCJ or SM and SBP were applied to ensile SBL at 10% and 20% (wet weight basis) levels, a better performance was observed in terms of silage quality and rumen fermentation parameters among the combinations. However, this combination may be at the risk of spoilage, and it must be less exposed to air.

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