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Partial replacement of corn silage with whole-plant soybean and black oat silages for dairy cows

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ABSTRACT

This study aimed to evaluate the effects of partially replacing corn silage (CS) with whole-plant soybean silage (SS) or black oat silage (OS) on nutrient intake and digestibility, in vitro neutral detergent fiber degradability of silages, feeding behavior, rumen fermentation, and performance of dairy cows. Twenty-four lactating Holstein cows (6 of which were rumen-cannulated) with 32.5 ± 4.92 kg/d milk yield, 150 ± 84.8 days in milk, and 644 ± 79.0 kg of body weight were used in a 3 × 3 Latin square design to evaluate the following treatments: (1) corn silage diet (CSD): using corn silage as the only forage source in the diet [48% dietary dry matter (DM)]; (2) whole-plant soybean silage diet (SSD): SS replacing 16% of corn silage from CSD; and (3) black oat silage diet (OSD): OS replacing 16% of corn silage from CSD. The inclusion of OS and SS decreased intakes of DM, organic matter, and crude protein. Corn silage had the greatest in vivo effective degradability of DM, and SS had the least effective degradability of neutral detergent fiber. The OSD treatment decreased milk and protein yields, whereas SSD increased rumen ammonia nitrogen concentration compared with the other diets. Cows fed OSD exhibited a greater preference for feed with small particles (<4 mm) compared with those fed SSD. Cows fed treatments containing either SS or OS at the expense of CS had increased rumination and chewing activities. Although replacing CS with OS and SS reduced feed intake, SS had no effect on productive performance of dairy cows.

Key words: forage, *Glycine max*, legume silage, grass silage

INTRODUCTION

Roughages are essential in diets of dairy cattle given their role in stimulating chewing, rumination, production of saliva, and being an energy source for rumen microorganisms (Tafaj et al., 2006). Corn is the most widely grown crop for silage on Brazilian dairy farms (Bernardes and do Rêgo, 2014); however, consistently cropping corn as a monoculture can negatively affect nutrient extraction from the soil and increase the odds of erosion (Ueno et al., 2011). Complementary seasonal crops such as black oat (winter crop) and whole-plant soybean (second summer crop) have been used as a tool to minimize land idleness (Ferrazza et al., 2013), to increase nutrient cycling and nitrogen fixation in the soil (Stagnari et al., 2017), and to maintain soil coverage throughout the year.

Although several studies have suggested that NDF content (69.9–74.5%) of black oat silage (**OS**) is the limiting factor for performance (Meinerz et al., 2011; Lehmen et al., 2014; Leão et al., 2017), Salgado et al. (2013) reported an increase of CP intake and a trend toward increased FCM yield without affecting DMI when including OS in diets of lactating cows, indicating that the previous results might be related to the fiber quality of tested forages.

Because whole-plant soybean silage (**SS**) has greater CP content than corn silage (**CS**; 134 vs. 83.9 g/kg of DM; Ghizzi et al., 2020), replacing CS with SS may reduce the use of soybean meal or other protein feeds in diets of lactating cows (Baghdadi et al., 2016). However, the quality of SS may limit the performance of dairy cows (Ghizzi et al., 2020). Ghizzi et al. (2020) found that replacing up to 50% of CS with SS linearly decreased milk yield without affecting feed efficiency (milk yield/DMI) and milk fat content of cows.

Despite the agronomical benefits of soybean and black oat crops, the literature lacks studies evaluating the partial inclusion of SS and OS in diets of dairy cows. We hypothesized that the partial replacement of

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CS with SS or OS would sustain intake and digestibility of nutrients, rumen fermentation, and productivity of dairy cows. This experiment was carried out to evaluate the effects of SS and OS on silage in vitro degradability, feed intake and digestibility, sorting index, feeding behavior, rumen fermentation, and milk yield and composition of cows.

MATERIALS AND METHODS

The experiment was conducted at the Dairy Cattle Research Laboratory of the Department of Animal Nutrition and Animal Production, Pirassununga, Brazil. Experimental procedures were approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Sciences of the University of São Paulo (protocol number 3302101018).

Animals and Treatments

Twenty-four Holstein cows (6 of which were rumen cannulated), with milk yield of 32.5 ± 4.92 kg/d, 150 ± 85 DIM, and 644 ± 79 kg of BW (mean ± SD), were blocked by milk yield and parity and randomly assigned to a treatment sequence in a 3 × 3 Latin square design balanced for carryover effects. The experimental treatments were (1) corn silage diet (**CSD**), using corn silage as the only forage source; (2) soybean silage diet (**SSD**), replacing corn silage with SS; and (3) oat silage diet (**OSD**), replacing corn silage with OS. The alternative silages replaced 16% of silage DM. Diets were formulated according to NRC (2001) and provided as TMR with a 48:52 forage-to-concentrate ratio (Table 1). The CS replacement rate was set based on results of a soybean silage study reported in Ghizzi et al. (2020), in which forage replacement rates >16% impaired productivity (milk yield and FCM) of cows according to the Tukey-Kramer honestly significant difference test. Experimental periods lasted 21 d, with 14 d for adaptation to diets and 7 d for sampling. Cows were housed in individual pens (17.5 m^2) containing fans, individual feed bunks, sand bedding, and free access to water.

Both SS (hybrid Agroeste 3610 I PRO, Agroeste) and CS (hybrid Biomatrix 3063, Agroceres) were produced at the Dairy Cattle Research Laboratory, whereas OS (hybrid IAPAR 61, Embrapa) was provided by Agropecuária Leffers. Chemical composition of silages are shown in Table 2.

Sampling

Cows were fed twice daily (0700 and 1300 h) and refusals targeted 5 to 10% (as-fed). Silages, TMR, and refusals were collected daily throughout the sampling periods, pooled into a composite sample per cow per period, and frozen (−20°C) for further analyses. Samples of ingredients in concentrate [ground corn, soybean meal, whole raw soybean, citrus pulp, bypass soybean meal (Soypass, Cargill), mineral premix, sodium bicarbonate, limestone, and salt] were collected once during each experimental period. Refusals and TMR were sampled on d 15 and 16 for particle size distribution (Penn State Particle Separator; Heinrichs and Kononoff, 2013), and sorting index was calculated according to Silveira et al. (2007). Fecal samples were collected every 9 h on d 18, 19, and 20 of each experi-

Table 1. Ingredients and chemical composition of the experimental diets (% of DM, unless otherwise stated)

	Diet[1]		
Item	CSD	OSD	SSD
Ingredient			
Corn silage	48.0	40.0	40.1
Black oat silage	—	8.00	—
Whole-plant soybean silage	—	—	8.01
Ground corn	18.3	18.7	19.6
Soybean meal	13.8	13.8	12.7
Citrus pulp	8.20	8.00	8.02
Whole raw soybean	6.47	6.41	6.44
Bypass soybean meal[2]	2.12	2.00	2.06
Mineral premix[3]	1.29	1.29	1.29
Sodium bicarbonate	0.83	0.81	0.80
Limestone	0.73	0.73	0.72
Salt	0.26	0.26	0.26
Chemical composition			
DM, g/kg, as-fed	42.6	40.4	40.8
NDF	34.4	35.3	33.8
NFC[4]	39.0	37.9	39.0
ADF	25.8	31.2	28.2
Forage NDF	27.0	27.8	26.5
Starch	19.0	16.5	17.2
CP	16.4	16.4	16.5
RDP[5]	10.4	10.4	10.5
RUP[6]	6.00	6.00	6.00
Ether extract	3.90	3.90	4.30
NE_L (3×) (Mcal/kg of DM)[7]	1.58	1.57	1.59
Particle size distribution (% as-fed)			
>19 mm	8.04	12.3	11.4
19–8 mm	36.0	34.0	37.2
8–4 mm	20.5	21.7	21.7
<4 mm	35.5	31.0	29.7

[1]The experimental diets were formulated according to NRC (2001) to meet the nutrient requirements of cows with 662 kg BW, 150 DIM, 32 kg/d milk yield, and 3.5% milk fat. CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]Soypass (Cargill).

[3]Each kilogram contained 235 g of Ca, 60 g of P, 20 g of Mg, 70 g of Na, 20g of S, 15 mg of Co, 700 mg of Cu, 10 mg of Cr, 40 mg of I, 600 mg of Fl, 1,600 mg of Mn, 20 mg of Se, 2,500 mg of Zn, 200,000 IU of vitamin A, 50,000 IU of vitamin D3, and 1,500 IU of vitamin E.

[4]Estimated according to Hall (2000).

[5]Estimated according to NRC (2001).

[6]Estimated according to NRC (2001).

[7]Estimated at 3 times the maintenance level according to NRC (2001).

mental period and frozen. At the end of each period, fecal samples were thawed and pooled into composite sample per cow.

On d 21 of each experimental period, samples of rumen content were collected from cannulated cows before (0) and at 2, 4, 6, 8, 10, 12, 14, and 16 h after the morning feeding. The rumen digesta was squeezed in 4 layers of cheesecloth to extract the rumen fluid. Afterward, the pH of rumen fluid was measured (MB-10, Marte Científica), and an aliquot (50 mL) from each time point was frozen for further analysis of ammonia nitrogen (NH_3-N) and short-chain fatty acids (**SCFA**).

Feeding behavior was monitored every 5 min between 0600 h of d 15 and 0600 h of d 17 in each experimental period for cows' activity (eating, drinking, standing, lying down, and milking times). Rumination activity was recorded using an electronic monitoring system (HealthyCow 24 Solution, SCR Allflex) in a collar attached to the cows. According to these activities, the index of rumination, idling [24 h − (eating + drinking + milking)], and chewing (rumination + eating) were calculated.

Cows were milked twice daily (0600 and 1600 h). Milk samples were collected from each cow (350 mL) for 3 consecutive days (15, 16, and 17 d) and analyzed fresh for contents of fat, protein, and lactose by mid-infrared methodology (Lactoscan, Entelbra, São Paulo, Brazil). The milk sampled on d 16 was deproteinized with trichloroacetic acid solution (25%) for MUN and allantoin analyses. Body condition score (Edmonson et al., 1989) and BW were measured on d 20 and 21 of each experimental period. Body weight was measured before the morning feeding and after milking using a digital scale (AWS100, DeLaval).

Blood samples were collected from tail vessels in Vacutainers without clot activator (BD Vacutainer, Becton, Dickinson and Co.) on d 17, 4 h after the morning feeding. Blood serum was collected after clot formation and centrifugation (2,000 × *g* for 15 min at room temperature) for further serum glucose analysis. Urine samples were collected at the same time points of fecal sampling. Immediately after collection, 20 mL of filtered urine was diluted in sulfuric acid solution (80 mL, 0.036 *N*) to prevent bacterial destruction of purine derivatives (**PD**) and uric acid precipitation (Chen and Gomes, 1992). Urine samples were then stored frozen until creatinine, allantoin, uric acid, and N analyses.

Table 2. Chemical composition of the experimental silages (% of DM, unless otherwise stated)

Chemical composition	Silage[1]		
	Corn	Black oat	Soybean
DM, % as-fed	27.6	22.6	23.5
OM	95.5	91.4	90.4
NDF	57.8	71.4	54.8
ADF	42.7	54.1	48.0
NFC[2]	27.0	12.3	18.4
Starch	20.5	16.4	15.6
iNDF[3]	21.5	30.6	31.1
Lignin	7.45	8.02	13.0
CP	8.48	5.61	12.6
NDIP[4]	1.41	1.07	2.63
ADIP[5]	1.10	0.91	25.1
Ether extract	2.19	2.06	4.40

[1]Corn: harvested at the phenological stage R5–beginning of the development of the milk line Ritchie et al. (1989). Black oat: harvested in a late cycle–from emergence to full bloom (Sá, 1995). Whole soybean plant: harvested at the phenological stage R5–beginning seeds (Fehr and Caviness, 1977).

[2]Estimated according to Hall (2000).

[3]Indigestible NDF.

[4]Neutral detergent insoluble protein.

[5]Acid detergent insoluble protein.

In Vitro Ruminal Degradability of Silages

Silage samples were collected on each experimental period, dried in a forced-air oven at 55°C for 24 h, and ground to pass through 2-mm sieve. Ground samples (600 mg) were placed in prewashed nonwoven tissue bags (25 µm porosity and 5 × 5 cm). One bag from each silage within each period (9 in total) were placed in bags of plastic nets (15 × 30 cm) for in vitro incubation. One plastic net bag for each time point (3, 8, 16, 24, 48, 72, and 96 h) of incubation was prepared and incubated in quadruplicate (one plastic net bag on each incubator jar). Periods were used as repetitions for statistical analysis.

To prepare the incubation medium, rumen digesta was collected from 4 cannulated cows previously adapted (14 d) to the CSD treatment. Samples of rumen fluid and solid phase (1:1, vol/vol) from the same animal were homogenized in a blender for 10 s and then filtered through 4 layers of cheesecloth (35 µm porosity; Bueno, 2002). After filtering, ruminal fluid (400 mL) was mixed with buffer and mineral solution (1.6 L) for incubation. The buffer and mineral solutions were prepared according to McDougall (1948) and Williams (2000). The previous solution remained under continuous CO_2 bubbling until no oxygen was detected, indicated by the color change of the liquid from blue to pink. Samples and incubation medium were placed in incubation jars and maintained in a Daisy in vitro system (Ankom Technology Corp.) at 9 rpm and 39°C. The incubation medium prepared from the rumen digesta of each cow was placed in different jars. One plastic net was removed from each incubation jar after 3, 8, 16, 24, 48, 72, and 96 h of incubation (Bueno, 2002). One plastic net with silage samples was submerged in

the incubation solution for 5 min to account for time point 0 (Benchaar et al., 2006).

Small bags were washed in running tap water, dried in a forced-air oven at 55°C for 24 h, and subsequently at 105°C for 2 h. Bags were then weighed and analyzed for DM (method 930.15; AOAC International, 2000) and NDF using α-amylase without sodium sulfite in the detergent solution (Undersander et al., 1992). Based on the disappearance of DM and NDF throughout the time points, rumen degradability (**DEG**) and effective degradability (**ED**) were estimated following the models proposed by Van Milgen et al. (1991) and Ørskov and McDonald (1979), respectively:

$$\text{DEG } (\text{g/kg}) = C + B \times (1 + \lambda \times t) \times \exp^{(-\lambda \times t)},$$

where C is the indigestible fraction, B is the potentially degradable fraction, λ is the constant rate of degradation, t is the time of incubation, and:

$$\text{ED } (\text{g/kg}) = 1{,}000 - \left[C + B\left(\frac{k_{p_b}}{k_{d_b} + k_p}\right)\right],$$

where k_{p_b} is the passage rate for fraction B, and k_{d_b} is the constant degradation rate of fraction B. Effective degradation was calculated considering 20, 50, and 70 g/kg per hour as passage rates (k_p); C and B were as previously described.

Chemical Analysis and Calculations

Feeds, refusals, and feces were dried in a forced-air oven at 55°C during 72 h, and ground in a knife mill (MA340, Marconi) to pass through 1-mm and 2-mm screens. Samples ground to pass a 1-mm screen were analyzed for DM (method 930.15), ash (method 942.05), OM (DM − ash), CP (method 984.13, N × 6.25, Kjeldahl method), lignin (method 973.18), and ether extract (**EE**; method 920.39) according to AOAC International (2000). The NDF content was analyzed according to Undersander et al. (1992), and ADF was analyzed according to Van Soest and Mason (1991). Fiber analyses were performed in a fiber analyzer (TE-149 fiber analyzer, Tecnal Equipamentos Científicos). Samples NFC content was calculated according to Hall et al. (2000), as follows: NFC = 1,000 – [(NDF – NDIN) × 6.25 + CP + EE + ash] with values expressed in grams per kilogram. Feed ingredients were analyzed for acid detergent insoluble protein and neutral detergent insoluble protein according to Licitra et al. (1996). Ingredients were also analyzed for starch through an enzymatic degradation method (Amyloglucosidase, Novozymes) and absorbance measured on a semi-automatic spectrophotometer (SBA-200, CELM) according to Hendrix (1993).

Samples ground to pass a 2-mm screen were placed (0.5 g) in 5 × 5 cm nonwoven fabric bags (20 mg of DM/cm^2 of surface; Casali et al., 2008) and incubated in the rumen of 2 cannulated cows acclimated to the CSD diet for 288 h (Huhtanen et al., 1994) to estimate the indigestible NDF. Indigestible NDF was used as an internal marker to estimate daily fecal excretion and to calculate the digestibility of DM and nutrients based on the following equations:

$$\text{DM digestibility } (\%) = 100 - \left[100 \times \left(\frac{\%\text{ iNDF intake}}{\%\text{ iNDF in feces}}\right)\right],$$

and

$$\text{Nutrient digestibility } (\%) = 100 - \left[100 \times \left(\frac{\%\text{ iNDF intake}}{\%\text{ iNDF in feces}}\right) \times \left(\frac{\%\text{ nutrient in feces}}{\%\text{ nutrient intake}}\right)\right].$$

Rumen fluid samples were centrifuged (2,000 × g for 15 min at room temperature), and 1.8 mL of the supernatant was pipetted into a centrifuge tube containing 400 μL of orthophosphoric acid solution (1 N) for SCFA analysis. Peaks of SCFA were measured on gas chromatograph (Shimadzu GC-2010 Plus) equipped with an automatic flame injector (AOC-20i, Stabilwax-DA 30-m capillary column, 0.25 mm internal diameter, 0.25-μm film thickness, Restek) and a flame ionization detector, as described by Del Valle et al. (2018). Another aliquot from the supernatant (800 μL) was mixed with sulfuric acid solution (400 μL at 1 N) for NH_3-N determination using the phenol-hypochlorite method (Broderick and Kang, 1980).

Daily urinary excretion was estimated based on the creatinine concentration in urine, considering a daily excretion of 0.212 mmol of creatinine per kg of BW as reported by Chizzotti et al. (2008). The creatinine and uric acid in urine were analyzed using commercial kits (Bioclin) and absorbances measured on a biochemistry analyzer (SBA-200, CELM). Allantoin concentrations in urine and milk were analyzed according to Chen and Gomes (1992) and absorbances measured on a microplate reader (Biochrom Asys UVM 340 Microplate Reader). The absorbed PD (mmol/d) was calculated based on the excretion of PD (mmol/d) in milk and urine, using the equation

$$\text{Absorbed PD} = \frac{\text{PD} - \left(0.512 \times \text{BW}^{0.75}\right)}{0.84},$$

where PD is the sum of excreted PD, 0.512 × $\text{BW}^{0.75}$ represents the endogenous excretion of PD, and 0.84 is the recovery of absorbed PD (Gonzalez-Ronquillo et al., 2003). Microbial protein synthesis (**MPS**; grams of N per day) was calculated as

$$\text{MPS } (\text{g/d}) = \frac{\left(70 \times \text{Absorbed PD}\right)}{\left(0.83 \times 0.116 \times 1{,}000\right)},$$

where 70 is N concentration in the PD (mg/mol), 0.83 is the intestinal digestibility of purines, and 0.116 is the purine-to-N ratio (Chen and Gomes, 1992).

The nitrogen balance was calculated as the difference between the total nitrogen intake (total N; CP intake/6.25) and the total nitrogen excreted in the feces (fecal N; fecal excretion × N fecal content), in the urine (urinary N; by method 984.13; AOAC International, 2000), and secreted in milk (milk N); milk yield × CP in milk/6.38). Serum glucose was determined by colorimetric biochemistry kits (glucose: cat. N. K-082, Bioclin) and absorbances measured on a semi-automatic spectrophotometer (SBA 200, CELM).

Statistical Analysis

Data were submitted to ANOVA using the PROC MIXED of SAS 9.4 (SAS, 2011) according to the following model:

$$Y_{ijkl} = \mu + Q_i + a_{j:i} + T_k + P_l + e_{ijkl},$$

with $a_{j:i} \approx N\left(0, \sigma_a^2\right)$ and $e_{ijkl} \approx N\left(0, \sigma_e^2\right)$, where Y_{ijkl} is the observed value of the response variable; μ is the overall mean; Q_i is the fixed effect of square ($i = 1$ to 8); $a_{j:i}$ is the random effect of animal within Latin square ($j = 1$ to 24); T_k is the fixed effect of treatment ($k = 1$ to 3); P_l is the fixed effect of experimental period ($l = 1$ to 3); e_{ijkl} is the experimental error; N indicates the normal distribution (Gaussian); σ_a^2 is the variance associated with the random animal effect; and σ_e^2 is the residual variance. The differences between treatments were estimated by the Tukey's range test.

Rumen fermentation data were analyzed according to the following model:

$$Y_{ijkl} = \mu + Q_i + a_{j:i} + T_k + P_l + \omega_{ijkl} + H_m + T \times H_{km} + e_{ijklm},$$

with $a_{j:i} \approx N\left(0, \sigma_a^2\right)$, $\omega_{ijkl} \approx N\left(0, \sigma_\omega^2\right)$, and $e_{ijklm} \approx MVN$ (0, **R**); where Y_{ijklm}, μ, Q_i, $a_{j:i}$, T_k, and P_l were previously described; ω_{ijkl} is the error associated with experimental units (animal within period); H_m is the fixed effect of the time ($m = 1$ to 9); $T \times H_{km}$ is the fixed effect of interaction between treatment and time; e_{ijklm} is the residual error; N indicates normal distribution (Gaussian); σ_a^2 is the variance associated with the random animal effect; σ_ω^2 is the residual variance associated with the animals in each evaluation period; MVN indicates multivariate normal distribution; and **R** is a matrix of variance. Different covariance structures due to repeated measures over time were tested [compound symmetry, heterogeneous compound symmetry, first-order autoregressive(1), heterogeneous AR(1), Toeplitz, heterogeneous TOEP, and unstructured] and chosen by the Bayesian criteria.

Average degradation from each experimental period (n = 3 per silage) were considered as repetitions in the statistical analysis of DEG and ED data using the PROC NLMIXED procedure of SAS 9.4. A single variance was used, and the estimate function was used to compare the coefficients. The differences between treatments were estimated using Fisher's LSD test. The statistical significance was considered when $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$ for all variables.

RESULTS

The SS had less ($P = 0.009$) B fraction in DM compared with CS and OS (Table 3). Corn silage had greater ($P < 0.001$) potentially digestible and C fractions in DM compared with OS and SS. Corn silage had the greateast ($P = 0.019$) k_{d_b} in DM, whereas OS had intermediate values, and SS the least value. Corn silage exhibited the greatest ($P < 0.001$) ED of DM, whereas SS had intermediate values, and OS the least values, regardless of k_p. The SS had lower ($P < 0.001$) content of B and C fractions, and ED of NDF (regardless of k_p) compared with CS and OS.

The inclusion of alternative silages (OS or SS) reduced ($P \leq 0.009$) the intake of DM, OM, and CP (Table 4). Cows fed SSD had lower ($P \leq 0.05$) NDF intake than those fed CSD. The OSD treatment reduced ($P \leq 0.05$) EE intake across diets. Cows fed OSD had greater ($P = 0.049$) preference for feed with small particles (<4 mm) than animals fed SSD.

Feeding SS or OS increased ($P \leq 0.029$) ruminating and chewing activities compared with CSD (Table 5). Regarding the activity measured relative to NDF intake (min/kg of NDF), animals fed SSD had greater ($P \leq$

Table 3. Rumen degradability of DM and NDF of experimental silages (mean ± SE)[1]

Item	Silage[2]			*P*-value
	CS	OS	SS	
DM degradability				
B fraction,[3] %	41.5[a] ± 2.10	39.3[a] ± 2.09	33.4[b] ± 1.49	0.009
Potentially digestible,[4] %	73.4[a] ± 1.90	57.4[b] ± 1.92	57.9[b] ± 1.49	<0.001
C fraction,[5] %	26.6[a] ± 1.89	42.6[b] ± 1.92	42.1[b] ± 1.49	<0.001
k_{d_b},[6] %	6.37[a] ± 0.663	6.49[ab] ± 0.749	8.57[b] ± 0.944	0.019
Effective degradability,[7] %				
k_p = 20 g/kg per hour	63.4[a] ± 1.08	48.2[c] ± 1.08	51.6[b] ± 1.01	<0.001
k_p = 50 g/kg per hour	55.1[a] ± 0.81	40.3[c] ± 0.81	45.6[b] ± 0.82	<0.001
k_p = 70 g/kg per hour	51.7[a] ± 0.79	37.0[c] ± 0.79	42.9[b] ± 0.79	<0.001
NDF degradability				
B fraction, %	61.6[a] ± 4.38	50.4[a] ± 5.09	37.1[b] ± 4.16	<0.001
C fraction, %	39.4[a] ± 4.02	41.2[a] ± 4.95	64.1[b] ± 3.57	<0.001
k_{d_b}, %	6.06 ± 0.865	4.96 ± 0.913	6.74 ± 1.47	0.314
Effective degradability, %				
k_p = 20 g/kg per hour	45.3[a] ± 2.22	4.44[a] ± 2.43	27.4[b] ± 2.14	<0.001
k_p = 50 g/kg per hour	32.8[a] ± 1.65	3.35[a] ± 1.66	20.1[b] ± 1.65	<0.001
k_p = 70 g/kg per hour	27.6[a] ± 1.59	29.3[a] ± 1.58	17.0[b] ± 1.60	<0.001

[a–c]Means within row with different superscripts differ significantly in the Fisher test (least significant difference, $P < 0.05$).

[1]Silage samples collected in each period (n = 3) were considered as repetitions in statistical analysis.

[2]CS: corn silage; OS: oat silage; SS: whole-plant soybean silage.

[3]B = potentially degradable insoluble fraction.

[4]Potentially degradable fraction (soluble and insoluble).

[5]C = indigestible fraction.

[6] k_{d_b} = degradation rate of fraction B.

[7]Effective degradability considering passage rates of 20, 50 and 70 g/kg per hour.

Table 4. Feed intake, total-tract apparent digestibility, and sorting index of lactating cows fed oat silage and soybean silage partially replacing corn silage in the diet (72 experimental units)

Item	Treatment[1]			SEM	*P*-value
	CSD	OSD	SSD		
Intake, kg/d					
DM	26.2[a]	25.3[b]	25.1[b]	0.65	0.009
OM	24.3[a]	23.1[b]	23.1[b]	0.57	<0.001
NDF	9.82[a]	9.67[ab]	9.47[b]	0.244	0.023
CP	4.65[a]	4.31[b]	4.34[b]	0.102	<0.001
Ether extract	0.91[a]	0.86[b]	0.90[a]	0.021	<0.001
Digestibility coefficient, %					
DM	67.7	66.5	67.4	0.65	0.371
OM	69.5	67.9	69.0	0.64	0.130
NDF	45.8	44.6	44.9	1.45	0.716
CP	70.9[x]	69.2[y]	70.4[xy]	0.64	0.069
Ether extract	83.8	81.5	83.1	0.99	0.164
Sorting index[2]					
>19 mm	0.991	1.004	0.999	0.007	0.417
19–8 mm	1.025	1.032	1.031	0.002	0.153
8–4 mm	1.033	1.034	1.034	0.003	0.942
<4 mm	1.054[ab]	1.059[a]	1.049[b]	0.002	0.049

[a,b]Means within rows with different superscripts differ significantly in Tukey's honestly significant difference test ($P < 0.05$).

[x,y]Means within rows with different superscripts tended to differ significantly in Tukey's honestly significant difference test ($P < 0.10$).

[1]CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]Values <1 indicate sorting against the particular size, values >1 indicate sorting for particles on the particular size range, and value = 1 indicates no sorting.

Table 5. Feeding behavior of lactating cows fed oat silage and soybean silage partially replacing corn silage in the diet (72 experimental units)

Item	Treatment[1]			SEM	*P*-value
	CSD	OSD	SSD		
Activity, min/d					
Drinking	25.8	26.8	24.6	2.57	0.804
Eating	309	316	317	7.94	0.668
Lying down	735	746	718	15.1	0.310
Ruminating	529[b]	554[a]	543[a]	12.6	<0.001
Idling[2]	935	925	928	8.63	0.636
Chewing[3]	839[b]	871[a]	860[a]	16.6	0.029
Activity min/kg of NDF intake					
Eating	32.1[y]	33.1[xy]	34.4[x]	1.39	0.099
Ruminating	54.9[b]	57.5[ab]	59.0[a]	1.84	0.002
Chewing	87.0[b]	90.6[ab]	93.4[a]	3.01	0.004

[a,b]Means within rows with different superscripts differ significantly in Tukey's honestly significant difference test ($P < 0.05$).

[x,y]Means within rows with different superscripts tended to differ significantly in Tukey's honestly significant difference test ($P < 0.10$).

[1]CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]Idling: 24 h – (drinking + eating + milking).

[3]Chewing = eating + ruminating.

0.004) ruminating and chewing activities and tended to exhibit greater ($P = 0.099$) time spent eating compared with animals fed CSD.

Cows fed OSD had higher ($P = 0.005$) rumen fluid pH than those fed CSD (Table 6). Cows fed SSD exhibited greater ($P \leq 0.05$) rumen NH_3-N concentration than those fed OSD. No evidence for treatment effects on rumen SCFA concentration was detected in this study.

Although cows fed OSD and SSD had lower ($P < 0.001$) N intake than those fed CSD, the excretion of N through feces and urine were not affected ($P \geq 0.157$) by treatments (Table 7). On the other hand, OSD cows had lower ($P \leq 0.014$) N secretion in milk than CSD. There was no evidence for treatment effects on MPS; however, we observed a tendency for greater ($P = 0.070$) efficiency of MPS for OSD treatment over SSD.

Cows fed OSD had lower ($P < 0.001$) yields of milk, protein, and lactose than those fed CSD and SSD (Table 8). Feeding OSD tended to decrease ($P \leq 0.062$) FCM in relation to CSD. In addition, OSD decreased ($P \leq 0.05$) milk production efficiency, and tended to increase ($P = 0.090$) milk fat content compared with SSD. Diets

Table 6. Rumen pH, NH_3-N concentration, and short-chain fatty acid (SCFA) profile of lactating cows fed oat silage and soybean silage partially replacing corn silage in the diet (18 experimental units)

Item	Treatment[1]			SEM	*P*-value		
	CSD	OSD	SSD		Trt	Time	Trt × Time[2]
pH	6.08[b]	6.21[a]	6.10[ab]	0.03	0.005	<0.001	0.828
NH_3-N, mg/dL	14.3[ab]	13.2[b]	17.1[a]	1.12	0.032	<0.001	0.087
SCFA, m*M*							
Acetate	71.9	68.8	70.5	2.84	0.410	<0.001	0.495
Propionate	24.0	22.4	23.4	1.26	0.317	<0.001	0.678
Butyrate	15.8	14.8	15.7	0.97	0.122	<0.001	0.632
BCFA[3]	4.74	4.76	5.07	0.27	0.378	0.003	0.441
Total SCFA	112	106	110	4.91	0.273	<0.001	0.549
Acetate to propionate ratio	3.04	3.10	3.07	0.07	0.665	0.065	0.336

[a,b]Means within rows with different superscripts differ significantly in Tukey's honestly significant difference test ($P < 0.05$).

[1]CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]Covariance matrices used in repeated measurements: heterogeneous TOEP (pH and acetate to propionate ratio), Toeplitz (NH_3-N), heterogeneous autoregressive(1) (acetate, propionate, butyrate, total SCFA), and compound symmetry (branched-chain fatty acids).

[3]Branched-chain fatty acids.

Table 7. Serum glucose, nitrogen balance, and microbial protein of lactating cows fed oat silage and soybean silage partially replacing corn silage in the diet (72 experimental units)

	Treatment[1]				
Item	CSD	OSD	SSD	SEM	*P*-value
N intake, g/d	744[a]	690[b]	694[b]	16.4	<0.001
Fecal N, g/d	200	199	193	7.31	0.523
Urinary N, g/d	179	180	160	11.4	0.157
Milk N, g/d	169[a]	159[b]	164[ab]	3.90	<0.001
N balance,[2] g/d	197[a]	153[b]	177[ab]	15.3	0.016
MUN, mg/dL	9.64	9.54	9.60	0.25	0.969
Glucose, mg/dL	57.1	57.0	56.7	0.93	0.923
Microbial protein, kg/d	1.76	1.76	1.63	0.11	0.445
Efficiency[3]	0.097[xy]	0.110[x]	0.090[y]	0.007	0.070

[a,b]Means within rows with different superscripts differ significantly in Tukey's honestly significant difference test ($P < 0.05$).

[x,y]Means within rows with different superscripts tended to differ significantly in Tukey's honestly significant difference test ($P < 0.10$).

[1]CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]Calculated as N intake (CP intake/6.25) – [(CP milk/6.38) + (N urine) + (Fecal excretion × N feces)].

[3]Microbial protein (kg/d)/digestible organic matter intake (kg/d). Digestible organic matter intake calculated according to the equation: OM intake – [Fecal production (kg of DM) × OM exceted feces/100] × 100/OM intake.

did not affect ($P \geq 0.283$) milk fat yield, milk protein or lactose contents, FCM efficiency, BCS, or BW.

DISCUSSION

We hypothesized that partially replacing CS with either OS or SS would have no effect on productivity of cows. Diets containing these forages, however, impaired DMI and OSD had lower milk yield compared with control. The possible reasons for lower DMI might be related to differences in chemical composition, fiber degradability, and particle size distribution among diets. As observed in the in vitro degradability trial, alternative silages had inferior DM ED compared with

Table 8. Milk yield and composition cows fed oat silage and soybean silage partially replacing corn silage in the diet (72 experimental units)

	Treatment[1]				
Item	CSD	OSD	SSD	SEM	*P*-value
Yield, kg/d					
Milk	32.9[a]	31.1[b]	32.1[a]	0.661	<0.001
FCM[2]	34.3[x]	33.0[y]	33.4[xy]	0.946	0.062
Fat	1.23	1.20	1.20	0.045	0.335
Protein	1.08[a]	1.01[b]	1.05[a]	0.025	<0.001
Lactose	1.61[a]	1.52[c]	1.56[b]	0.018	<0.001
Milk composition, %					
Fat	3.76[xy]	3.87[x]	3.72[y]	0.107	0.090
Protein	3.28	3.27	3.27	0.024	0.705
Lactose	4.91	4.90	4.88	0.036	0.283
Efficiency					
Milk yield/DMI	1.26[ab]	1.24[b]	1.28[a]	0.015	0.046
FCM/DMI	1.31	1.31	1.32	0.027	0.681
BCS, 1–5	2.52	2.47	2.51	0.043	0.410
BW, kg	657	656	655	12.7	0.934

[a–c]Means within rows with different superscripts differ significantly in Tukey's honestly significant difference test ($P < 0.05$).

[x,y]Means within rows with different superscripts tended to differ significantly in Tukey's honestly significant difference test ($P < 0.10$).

[1]CSD: corn silage (CS)-based diet; OSD: replacement of CS with oat silage at 16.0%; and SSD: replacement of CS with soybean silage at 16.0%.

[2]3.5% FCM = (0.432 + 0.165 × milk fat percentage) × milk yield (kg/d).

CS. The DM digestibility of SS and OS varies in the literature (Vargas-Bello-Pérez et al., 2008; Restelatto et al., 2013; Baghdadi et al., 2016; Bueno et al., 2020) where differences are strongly related to the chemical composition of the forage, especially with contents of NDF and lignin (Kawamoto et al., 2013). In this study, the relatively high concentrations of NDF, ADF, indigestible NDF, and lignin in oat silage and soybean silage likely impaired degradation and nutritive value compared with corn silage. In addition, for optimal feed preservation during the ensiling process, pre-ensiled material must have an ideal DM content, water-soluble carbohydrate, and low buffering capacity to achieve a low pH (McDonald, 1991), which are not optimal in the legume forages used in this study.

Despite the lower in vitro ED of DM and NDF observed in OS and SS, treatments did not affect the total-tract apparent digestibility of nutrients. Cows fed OSD and SSD had lower feed intake, which often decreases the passage rate throughout the gut, increasing the retention time in the rumen and feed degradation. In addition, OSD and SSD treatments had greater proportion of feed with longer particles (>19 mm) in the diet, which might have contributed to slowdown the digesta and decrease the feed intake. Supporting the results of this study, other trials have reported an increase in chewing and ruminating activities when feeding silages of oat–ryegrass or SS (Celis-Alvarez et al., 2016; Ghizzi et al., 2020). Feeding activities are closely related to the NDF content and digestibility (Zebeli et al., 2006). Soybean silage used in this study exhibited relatively low value of NDF ED (27.4%), presenting values even lower than those (31.4%) reported by Vargas-Bello-Pérez et al. (2008). The lignin content and the extensive cross linking between lignin and carbohydrates in the soybean plant cell wall compared with the other silages (Mustafa et al., 2007) may decrease the ruminal ED of NDF.

In the current study, OSD decreased milk yield relative to CSD and SSD. Dairy cows are significantly affected by roughage DM and NDF digestibility as poor fiber digestibility reduces feed intake and milk yield (Zebeli et al., 2012). Oba and Allen (1999) reported that a 1-unit increase in NDF digestibility (in vitro or in situ) leads to a 0.17-kg increase in DMI and a 0.25-kg increase in 4% FCM. The trend toward a decreased CP digestibility in cows fed OSD could also have contributed to reducing milk yield. The histochemical and structural variations of black oat, when compared with corn, have a significant effect in the digestion process and absorption of nutrients (Elizalde et al., 1992). The accessibility of rumen microorganisms to oat fiber carbohydrates is limited due to the structural arrangement of their cells and the chemical composition of the cell wall, influencing the physical degradation, passage rate, and intake of forage (Abeysekara, 2003). According to Phillips et al. (1996), the performance of cattle fed OS is usually worse than the result achieved by cattle fed CS due to relatively low digestibility of NDF.

Although Ghizzi et al. (2020) reported a linear decrease in productivity of dairy cows when replacing CS with SS up to 50% diet DM, authors did not observe differences in milk and FCM yields between control and the lowest level of CS replacement with SS when data were submitted to the Tukey-Kramer honestly significant difference test. Similarly to this study, when authors fed SS at 16.7% there was no negative effect on feed efficiency of cows with an average milk yield of 33 kg/d (Ghizzi et al., 2020). Thus, in addition to the silage quality, the inclusion level may be a key point to optimize SS and OS utilization in dairy cow diets. In addition, the greater EE intake of cows fed SSD in relation to cows under CSD treatment might have offset the lower DM intake, thus maintaining similar milk yield between treatments. The greatest CS ruminal ED, followed by SS and lastly by OS, as well as greater feed intake for animals fed CSD, resulted in different amounts of available nutrients and consequently, differences in yields of protein and lactose.

The lower in vitro DM degradability in OS and the greater physically effective NDF of OSD are likely responsible for higher rumen pH. Furthermore, cows fed SSD showed a greater rumen concentration of NH_3-N compared with OSD. Aligning with results observed in this study, Ghizzi et al. (2020) reported a linear increase in ruminal NH_3-N when cows were fed SSD partially because of a decrease in DMI and fiber digestibility. A decrease in DMI reflects in less substrate for fiber-degrading bacteria which use rumen NH_3-N as nitrogen source for growth (Russell et al., 1992). Calsamiglia et al. (2008) observed an adverse effect of lower ruminal pH on the efficiency of microbial protein synthesis. Although CSD decreased ruminal pH in the present study, microbial protein synthesis did not differ among treatments.

CONCLUSIONS

Soybean and oat silages used herein had lower in vitro DM degradability in comparison with CS. Partial replacement of corn silage with OS or SS (8% diet DM) decreased DMI without affecting total-tract nutrient digestibility. Cows fed SSD, however, had similar milk yield and greater rumen pH in comparison with CSD cows. Soybean silage may partially replace CS in diets of lactation without milk production losses.

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