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The effect of length of storage and sodium benzoate on the nutritive value of reconstituted sorghum grain silages for dairy cows

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ABSTRACT

Twenty Holstein cows at 168 ± 87 d in milk (mean ± SD) were assigned to a 4 × 4 Latin square design with a 2 × 2 factorial arrangement to evaluate the effects of 2 storage lengths (30 or 90 d) and the presence of sodium benzoate (control or 0.2% as fed) on the nutritive value of reconstituted sorghum grain silages (RSGS). For each treatment, dry ground sorghum grain was rehydrated to 35% moisture and ensiled in 200-L plastic drums. The treatments were RSGS stored for 30 d without sodium benzoate (30 CON), RSGS stored for 30 d with sodium benzoate (30 BEN), RSGS stored for 90 d without sodium benzoate (90 CON) and RSGS stored for 90 d with sodium benzoate (90 BEN). Diets contained 16.3% RSGS. Silages stored for 90 d had higher concentrations of 1,2-propanediol, soluble protein, and ammonia nitrogen than did those stored for 30 d. Sodium benzoate reduced ethanol and ethyl-ester formation. Silages stored for 90 d had higher starch (89.3 vs. 86.9%) and protein (57.1 vs. 54.0%) digestibility compared with silages stored for 30 d. The ruminal acetate-to-propionate ratio tended to be lower in RSGS stored for 90 d than in RSGS stored for 30 d (3.75 vs. 3.34). Milk yield increased from 30.0 kg/d in cows fed RSGS stored for 30 d to 31.2 kg/d in cows fed RSGS stored for 90 d, without a change in dry matter intake (23.5 kg/d on average). Hence, feed efficiency and milk N efficiency also had tendencies to increase in cows fed RSGS stored for 90 d. Sodium benzoate did not alter cow performance but slightly increased plasma glucose (65.2 vs. 63.6 mg/dL). In conclusion, increasing the storage period of RSGS from 30 to 90 d improved starch and protein digestibility, milk yield, and feed efficiency.

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Key words: starch digestibility, chemical additive, proteolysis

INTRODUCTION

Ensiling cereal grains such as sorghum and corn has been associated with increased starch digestibility in ruminants (Owens et al., 1986). Replacing dry ground feed with high-moisture cereal grain silage often increases feed efficiency by increasing milk yield without affecting DMI (Oba and Allen, 2003; Arcari et al., 2016) or by decreasing DMI without altering milk yield (Ferraretto et al., 2013).

The breakdown of the protein matrix is considered the main reason for the increase in starch digestibility in grain silages (McAllister et al., 1993). Plant and microbial enzymes are the main contributors to proteolysis during silage fermentation (Junges et al., 2017), and a minimum length of storage is required to improve starch digestibility (Benton et al., 2005). It is well established in the literature that lengthening silage storage increases proteolysis and, consequently, starch digestibility (Der Bedrosian et al., 2012; Kung et al., 2018). However, to our knowledge, in vivo studies evaluating the performance of dairy cows fed high-moisture grain silages after different lengths of storage have not been performed.

Sodium benzoate is a chemical additive that is highly efficient at increasing aerobic stability (Morais et al., 2017). However, sodium benzoate has been associated with a reduction in proteolysis during silage fermentation (Da Silva et al., 2015). Therefore, we hypothesized that storing reconstituted sorghum grain silages (**RSGS**) for at least 60 d longer than silages stored for 30 d would increase starch digestibility and feed efficiency in dairy cows, whereas the addition of sodium benzoate might reduce proteolysis during silage fermentation and impair starch digestibility. The objective of this experiment was to evaluate the effect of RSGS storage for 30 or 90 d and treatment with or without

sodium benzoate on silage conservation, rumen fermentation, starch digestibility, and dairy cow performance.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics Committee for Animal Use of the University of São Paulo (protocol 2017.5442.11.4).

Ensiling

A grain sorghum hybrid (BM 737, Biomatrix, Rio Claro, Brazil) was sown during the second crop season in February 2016. Grains with 12% moisture were mechanically harvested (MF 3640, Massey Ferguson, Duluth, GA) at the end of July and stored in a metal bin with 12% moisture (Kepler Weber, Campo Grande, Brazil).

Ensiling was scheduled to allow for 2 different lengths of storage (30 and 90 d) at the time of silo opening. Silages stored for 90 d were prepared 60 d before silages stored for 30 d. Before making the silage, sorghum DM was determined by drying in an oven at 105°C for 12 h, and the result was used to calculate the amount of water needed to reach the target moisture content (35%). Before ensiling, grains were ground with a hammer mill through a 2-mm sieve. Dry ground sorghum was mixed with water in a vertical feed wagon (VM4; DeLaval, Tumba, Sweden) for 15 min. Fifteen hundred kilograms of silage was prepared per treatment for each experimental period and ensiled in six 200-L plastic drums. The silage density was 1,100 ± 50 kg/m^3 (±SD) as fed.

Sodium benzoate (number 532-32-1, Haofei Chemical, Zhengzhou, China) was used as the second study factor (0.2% of as-fed material) and was combined in a factorial arrangement with the length of storage. Sodium benzoate was diluted in water (1:130 wt/vol) for reconstitution before it was added to the feed material. Ensiling conditions were reproduced 4 times at 21-d intervals to ensure that the cows received silages stored for the same length of time in all 4 experimental periods. Each ensiling period was considered to be 1 block in the statistical analyses of silage outcomes.

Feeding Trial

Twenty Holstein cows (16 multiparous and 4 primiparous) were housed in a freestall barn with sand beds and an individual feed monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil) validated by Chizzotti et al. (2015). At the beginning of the trial, cows were 168 ± 87.4 DIM, with a milk yield of 32.3 ± 5.3 kg/d and BW of 681 ± 47 kg (mean ± SD). Cows were grouped by parity and milk yield. One group was composed of 4 multiparous rumen-cannulated cows in late lactation (288 ± 10 DIM and milk yield of 21.0 ± 2.0 kg/d) to evaluate ruminal parameters. This group was also included in the data set of all other measurements. Cows were randomly assigned to the sequence of 4 treatments in a 2 × 2 factorial arrangement in a 4 × 4 Latin square design balanced for carryover effect with 21-d periods (15 d of adaptation + 6 d of sampling). The treatments were as follows: silage stored for 30 d without addition of sodium benzoate (**30 CON**); silage stored for 30 d with sodium benzoate added (**30 BEN**); silage stored for 90 d without sodium benzoate (**90 CON**); and silage stored for 90 d with sodium benzoate (**90 BEN**). Cows were fed (0600 and 1700 h) and milked (0600 and 1800 h) twice daily. Ration ingredients were mixed for 10 min in a pull-type vertical mixer wagon and delivered in amounts to allow 5 to 10% of orts.

Laboratory Analysis

From d 16 to 20 of each experimental period, samples of each diet ingredient and orts were collected daily and frozen to form a composite sample per period. These samples were dried in a forced-air oven for 72 h at 55°C and ground through a 1-mm mesh screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). Subsamples were analyzed for DM, ether extract (EE), and ash according to the Association of Official Analytical Chemists (AOAC, 1990; methods 934.01, 920.39, and 942.05, respectively). The NDF (expressed inclusive of residual ash, assayed with sodium sulfite and heat-stable amylase) was analyzed using a TE-149 Fiber Analyzer (Tecnal Equipamentos, Piracicaba, Brazil). The CP was analyzed using the Dumas method 990.03 (Wiles et al., 1998; FP-2000A Nitrogen Analyzer, Leco Corp., St. Joseph, MI). The NFC was calculated as NFC = 100 − (CP + ether extract + ash + NDF). Starch content was analyzed according to Hall (2009). Indigestible NDF (**iNDF**) was measured by ruminal in situ incubation for 288 h (Huhtanen et al., 1994). The nutrient composition of the consumed diets was calculated by dividing daily nutrient intake by DMI (Table 1). The DMI was determined as the difference between the amount of offered diet and orts (DM basis) during d 16 to 20 of each period.

Subsamples (25 g of fresh material) of RSGS were added to 225 g of deionized water and mixed for 4 min in a stomacher. The extract was filtered with 3 layers of cheesecloth and centrifuged at 10,000 × *g* for 15 min at −4°C. The supernatant was used to quantify ammonia nitrogen using the method of Chaney and Marbach (1962) adapted by Weatherburn (1967) and to quantify lactic acid (Pryce, 1969). The concentra-

Table 1. Ingredients and nutrient composition (mean ± SD) of the experimental diets with reconstituted sorghum grain silage (RSGS) stored for 30 or 90 d without (CON) or with (BEN) addition of sodium benzoate

	30 d		90 d	
Item	CON	BEN	CON	BEN
Ingredients, % of diet DM				
Corn silage	49.4 ± 0.73	49.3 ± 0.75	49.3 ± 0.69	49.3 ± 0.79
RSGS	16.2 ± 0.07	16.4 ± 0.16	16.3 ± 0.13	16.3 ± 0.21
Dry ground sorghum	13.8 ± 0.39	13.8 ± 0.39	13.8 ± 0.41	13.8 ± 0.38
Soybean meal	14.8 ± 0.26	14.8 ± 0.25	14.8 ± 0.27	14.8 ± 0.24
Rumen-protected soybean meal[1]	1.9 ± 0.03	1.9 ± 0.03	1.9 ± 0.04	1.9 ± 0.03
Urea	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01
Calcium soap of palm oil	1.2 ± 0.02	1.2 ± 0.02	1.2 ± 0.02	1.2 ± 0.02
Mineral mix	2.2 ± 0.04	2.2 ± 0.04	2.2 ± 0.04	2.2 ± 0.04
DM, % as fed	51.1 ± 0.75	51.1 ± 0.73	51.1 ± 0.78	51.1 ± 0.68
Nutrients, % of DM				
CP	16.8 ± 0.19	16.8 ± 0.09	16.8 ± 0.11	16.7 ± 0.26
NDF	34.9 ± 0.7	35.2 ± 0.9	34.4 ± 1.1	34.2 ± 0.7
Ether extract	3.3 ± 0.06	3.3 ± 0.04	3.3 ± 0.04	3.3 ± 0.03
Ash	6.1 ± 0.47	6.1 ± 0.41	6.1 ± 0.41	6.1 ± 0.42
NFC	38.9 ± 1.3	38.6 ± 1.6	39.5 ± 1.6	39.6 ± 1.1
Starch	31.8 ± 1.16	32.6 ± 0.85	32.1 ± 1.14	32.9 ± 1.20
Starch origin, % of total				
Corn silage	37.1 ± 1.1	37.0 ± 1.2	37.0 ± 1.1	37.0 ± 1.3
RSGS	34.0 ± 1.0	34.3 ± 1.1	34.2 ± 0.7	34.3 ± 1.2
Dry ground sorghum	27.4 ± 1.1	27.3 ± 1.1	27.3 ± 1.1	27.3 ± 1.0
Particle size distribution, % as fed				
>19 mm	2.7 ± 0.84	2.8 ± 1.42	2.7 ± 0.88	2.8 ± 1.64
8–19 mm	22.8 ± 2.87	22.9 ± 3.80	23.3 ± 2.07	23.4 ± 2.07
<8 mm	74.5 ± 3.64	74.0 ± 5.08	73.9 ± 2.94	73.8 ± 3.57

[1]Soypass, Borregaard LignoTech, Fernandina Beach, FL.

tions of VFA, alcohols, and esters were analyzed using a gas chromatographer with a mass detector (GCMS QP 2010 Plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m in length, 0.25 mm in internal diameter, and 0.25 μm in film thickness). The DM content was corrected for volatiles according to Weissbach (2009).

The ruminal gas production profile was measured using the methodology of Theodorou et al. (1994) as modified by Mauricio et al. (1999). In all assays, 1.0 g of dried sample was incubated in duplicate for 72 h in a water bath at 39°C. Pressure measurements were made at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60, and 72 h after inoculation, using a pressure transducer and data logger (PDL200, LANA/CENA-USP, Piracicaba, Brazil). Ruminal fluid was collected from 2 cannulated nonlactating Holstein cows fed corn silage and 3 kg of concentrate mixture (50% dry ground sorghum, 43% soybean meal, 3% urea, and 4% mineral and vitamin mix). Ruminal digesta was collected from the solid and liquid phases. The solid phase was collected from the dorsal sac by hand and squeezed. The liquid phase was collected from the ventral sac using a stainless steel probe. Fluids were placed separately in prewarmed (approximately 39°C) flasks and transported immediately to the laboratory. Equal volumes of solid and liquid phases were mixed for approximately 10 s, filtered through a 35-μm nylon filter, and kept in a water bath (39°C) with CO_2 until inoculation. Gas volume production was expressed as milliliters per gram of DM. A 1-pool exponential equation $V_t = V_f\,(1 - e^{-k(t - L)})$ with a discrete lag period was fitted to the data (Schofield et al., 1994) to estimate the following parameters: volume of gas at time t (V_t), final asymptotic gas volume (V_f), fractional rate of gas production (k), and lag time (L).

Particle size distribution was measured using a Ro-Tap Shaker (Bertel Ltda., Caieiras, Brazil) with 5 sieves with nominal square apertures of 4.75, 2.36, 1.70, 1.18, and 0.6 mm and a bottom pan. Approximately 500 g of sample, dried at 55°C for 72 h in a forced-air oven, was used to perform the analyses.

Milk yield was measured from d 16 to 20, and samples for milk composition were collected on d 17 and 19 of each period in flasks with bronopol. Milk was analyzed for fat, protein, lactose, and urea N by Fourier transform mid-infrared spectroscopy (Clínica do Leite, Piracicaba, Brazil). The ECM was calculated as milk energy secretion (NE_L, Mcal/kg = 0.0929 × Fat % + 0.0547 × CP % + 0.0395 × Lactose %) divided by 0.70 (assuming 0.70 Mcal/kg for milk with 3.7% fat, 3.2% protein, and 4.6% lactose). Three measures of efficiency were calculated: actual milk divided by DMI; ECM divided by digestible OM intake; and milk N secretion divided by N intake.

The total-tract apparent digestibility of DM, OM, NDF, CP, and starch was estimated using iNDF as a marker. Fecal grab samples were collected every 8 h from d 18 to 20 of each period from each cow. Samples were dried and analyzed for DM, NDF, starch, CP, ash, and iNDF as previously described. Total-tract apparent digestibility was calculated according to the following equation: 100 − (TMR iNDF/fecal iNDF) × (fecal nutrient concentration/TMR nutrient concentration).

Chewing activity was evaluated on d 18 of each period by visual observation of eating and ruminating, at 10-min intervals for 24 h. Sorting behavior was measured on the same day, according to the methodology of Leonardi and Armentano (2003). Particle size distribution of the offered diets and orts were measured using 2 sieves, 19 and 8 mm, and the bottom pan of the Penn State Particle Separator (Nasco, Fort Atkinson, WI; Lammers et al., 1996).

Blood samples were obtained on d 21 of each period from coccygeal vessels before the morning feeding and 1, 2, 3, 6, and 12 h after feeding. Vacutainer tubes containing potassium EDTA were used to collect samples for plasma urea nitrogen (**PUN**) analysis, and tubes with fluoride were used to collect samples for glucose analyses. After sampling, blood was immediately centrifuged for 20 min at 2,000 × *g* at room temperature (~21°C), and the plasma was frozen at –20°C. The PUN and glucose were analyzed in the laboratory using the Urea 500 and Glucose Enzimática Líquida kits (Doles Reagentes Para Laboratórios Ltda., Goiânia, Brazil), respectively.

Ruminal samples were obtained on d 16 and 17 of each period from rumen-cannulated cows every 3 h for 24 h after the morning feeding. Samples of the solid phase were manually separated into 4 different portions of the ventral rumen and squeezed through a cheesecloth into a beaker. The pH was immediately measured, and 100 mL of fluid was frozen in liquid N for 1 min and stored at −20°C. Analyses of ammonia nitrogen and VFA were performed as described for the silages.

Statistical Analyses

Statistical analyses were performed using PROC MIXED of SAS (version 9.3; SAS Institute Inc., Cary, NC). The RSGS data were analyzed as a randomized block design using the following model: $Y_{ijk} = \mu + B_i + SL_j + SB_k + SL_jSB_k + e_{ijk}$, where μ = overall mean, B_i = random effect of block (i = 1 to 4), SL_j = fixed effect of storage length (j = 30 or 90 d), SB_k = fixed effect of sodium benzoate (k = BEN or CON), SL_jSB_k = interaction between SL and SB, and e_{ijk} = residual error.

Cow variables were analyzed using the following model: $Y_{ijklm} = \mu + S_i + C_{j(i)} + P_k + SL_l + SB_m + SL_lSB_m + e_{ijklm}$, where μ = overall mean, S_i = fixed effect of Latin square (i = 1 to 5), $C_{j(i)}$ = random effect of cow within square (j = 1 to 20), P_k = fixed effect of period (k = 1 to 4), SL_l = fixed effect of storage length (l = 30 or 90), SB_m = fixed effect of sodium benzoate (m = BEN or CON), SL_lSB_m = interaction between SL and SB, and e_{ijklm} = residual error. Data collected over time (glucose and PUN) were analyzed as repeated measures using the same model including the effect of time and their interaction. The mean square of cow, period, and treatment factors was used as the error to test the treatment effects. Data obtained from rumen-cannulated cows were analyzed as repeated measures using the following model: $Y_{ijklm} = \mu + C_i + P_j + SL_k + SB_l + SL_kSB_l + H + SL_kH + SB_lH + T_kA_lH + e_{ijkl}$, where μ = overall mean, C_i = random effect of cow (i = 1 to 4), P_j = fixed effect of period (j = 1 to 4), SL_k = fixed effect of storage length (k = 30 or 90 d), SB_l = fixed effect of sodium benzoate (l = BEN or CON), SL_kSB_l = interaction between SL and SB, H = fixed effect of hour, SL_kH = interaction between SL and hour, SB_lH = interaction between SB and hour, T_kA_lH = interaction between SL, SB, and hour, and e_{ijkl} = residual error. Degrees of freedom were adjusted using the Kenward-Roger method. The covariance structure was chosen based on Akaike's information criterion among variance component structure (VC), compound symmetry (CS), autoregressive (Lag 1) structure (AR(1)) and unstructured covariance structure (UN). Differences between main effects were considered significant when $P \leq 0.05$ and trends when $P > 0.05$ and ≤ 0.10. One cow was removed from the trial in the fourth period because of mastitis. The remaining 19 cows completed the experiment.

RESULTS

Silages

Before ensiling, the DM content of ground sorghum was 87.8%. The moisture target for ensiling was 35%. Silage moisture ranged from 35.0 to 36.1%, and there was no difference ($P > 0.10$) in the DM content among the treatments (Table 2). Ammonia nitrogen (30 d = 3.44% of N, vs. 90 d = 4.89% of N) and soluble protein (30 d = 13.6% of CP, vs. 90 d = 20.9% of CP) increased by 53.6 and 41.8%, respectively ($P < 0.05$), with increasing storage length. Before ensiling, ammonia nitrogen and soluble protein of sorghum grain were 0.58% N and 8.5% CP, respectively. Sorghum grain silage stored for 30 d had 62.5% more soluble protein

Table 2. Composition, fermentation profile, and in vitro gas production of reconstituted sorghum grain, ensiled for 30 or 90 d, without (CON) or with (BEN) addition of sodium benzoate

	30 d		90 d			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	SL	SB	SL × SB
Nutrient								
DM, % as fed	63.9	65.0	64.2	64.5	0.90	0.93	0.14	0.35
Soluble CP, % of CP	14.3	12.9	23.5	18.3	2.61	0.03	0.25	0.50
NH_3-N, % of N	3.68	3.20	5.08	4.69	0.473	<0.01	0.21	0.88
Starch, % of DM	71.6	70.3	69.6	69.3	1.42	0.29	0.55	0.72
Fermentation profile								
pH	4.01	3.98	4.02	4.06	0.04	0.41	0.75	0.42
Lactic acid, % of DM	2.33	2.22	2.30	2.24	0.260	0.98	0.52	0.85
Acetic acid, % of DM	0.20	0.21	0.29	0.22	0.067	0.41	0.58	0.47
Ethanol, % of DM	0.24	0.10	0.16	0.07	0.039	0.20	0.01	0.57
1,2-Propanediol, mg/kg of DM	39	51	202	106	51.9	0.04	0.37	0.26
2,3-Butanediol, mg/kg of DM	104	65	97	55	23.4	0.70	0.08	0.94
Propionic acid, mg/kg of DM	14	20	28	39	9.6	0.10	0.37	0.75
Ethyl lactate, mg/kg of DM	65	32	81	33	9.4	0.37	<0.01	0.42
Ethyl acetate, mg/kg of DM	35	13	23	8	7.3	0.21	0.02	0.62
Butyric acid, mg/kg of DM	3.2	4.7	3.5	3.3	1.25	0.66	0.62	0.53
1-Propanol, mg/kg of DM	4.3	1.2	16.3	10.0	5.15	0.10	0.41	0.79
In vitro gas production								
Lag time, h	3.4	3.1	3.0	3.2	0.14	0.45	0.95	0.12
Fractional rate of gas production, %/ h	3.9	3.6	4.6	4.9	0.37	0.03	0.85	0.35
Cumulative gas production, mL/g of DM	260	263	255	264	5.21	0.68	0.22	0.50

[1]Probabilities for the effects of storage length (SL), sodium benzoate (SB), and the interaction between storage length and sodium benzoate (SL × SB). Significant differences when $P \leq 0.05$, and trends when $P > 0.05$ and ≤ 0.10.

than did nonfermented sorghum, whereas silages stored for 90 d had 146% more soluble protein than did nonfermented sorghum. Sodium benzoate did not alter ($P > 0.10$) protein solubility (CON = 18.9% of CP, vs. BEN = 15.6% of CP) or ammonia concentration (CON = 4.38% of N, vs. BEN = 3.95% of N).

The addition of sodium benzoate reduced ($P < 0.05$) ethanol concentration by 46% (CON = 0.15% of DM, vs. BEN = 0.08% of DM), the ethyl lactate concentration by 56% (CON = 73 mg/kg of DM, vs. BEN = 32 mg/kg of DM), and the ethyl acetate concentration by 63% (CON = 29 mg/kg of DM, vs. BEN = 10.5 mg/kg of DM) compared with nontreated silages. When silages were stored for 90 d, the concentration of 1,2-propanediol was increased ($P = 0.04$), and the propionic acid concentration tended to be twice as high ($P = 0.10$) compared with silages stored for 30 d (Table 2). The concentrations of lactic and acetic acid were not altered by the treatments and averaged 2.27 ± 0.04 and 0.23 ± 0.03% of DM (mean ± SD), respectively.

Before ensiling, dry ground sorghum had a geometric mean particle size of 890 ± 41.6 μm and fractional rate of gas production of 3.6%/h. The particle size was not altered by the treatments after ensiling and averaged 874 ± 7.2 μm ($P = 0.75$). The rate of gas production was increased by 21.1% (3.75 vs. 4.75%/h) in silages stored for 90 d compared with silages stored for 30 d ($P = 0.03$). We found no effect of treatment ($P > 0.10$) on lag time or cumulative gas production, which averaged 3.2 ± 0.12 h and 260 ± 3.0 mL/g of DM, respectively. Sodium benzoate did not alter the kinetics of gas production.

Animal Performance

Intake of DM and digestible OM were not altered by the treatments ($P > 0.10$) and averaged 23.4 ± 0.32 and 13.5 ± 0.18 kg/d, respectively (Table 3). Analysis revealed a tendency to increase milk yield ($P = 0.10$) and ECM ($P = 0.07$) by 1.2 kg/d when cows received diets containing the silage stored for 90 d compared with diets containing the silage stored for 30 d. Treatment with the silages stored for 90 d tended ($P = 0.10$) to increase feed efficiency, as calculated by milk yield/DMI, by 4.7% (30 d = 1.27 vs. 90 d = 1.33) and to increase the ratio of ECM to digestible organic matter intake (DOMI) by 4.8% (30 d = 2.16 vs. 90 d = 2.27). Although the components of milk were not different among treatments, longer storage increased ($P \leq 0.05$) the daily secretion of fat, protein, and lactose due to increased milk production upon feeding cows the silage stored for 90 d. The efficiency of conversion of nitrogen from the ration into milk nitrogen tended to increase by 5.4% (30 d = 24.1 vs. 90 d = 25.4; $P = 0.07$). The MUN concentration (30 d = 14.7 mg/dL vs. 90 d = 13.8 mg/dL; $P = 0.10$) was lower in cows fed diets containing silages stored for 90 d compared with those fed silages stored for 30 d. Nevertheless, daily secretion of MUN

was not altered by the treatments (Table 3). Sodium benzoate did not alter milk yield or milk composition.

The NDF intake (8.2 ± 0.25 kg/d) and digestibility of NDF (29.2 ± 0.66%) did not differ among the treatments (Table 4). Compared with cows fed diets containing silages stored for 30 d, cows fed diets with silages stored for 90 d exhibited increased total-tract starch digestibility (30 d = 86.9% vs. 90 d = 89.3%; $P = 0.04$) and a trend ($P = 0.06$) toward reduced fecal starch concentration (30 d = 9.3% vs. 90 d = 7.9%). The digestibility of CP and DM also tended ($P = 0.10$) to be higher when cows received diets containing silages stored for 90 d than when cows were fed diets containing silages stored for 30 d (Table 4). The sorting index and chewing behavior were not affected by the treatments (Table 4). Cows fed silages stored for 90 d tended ($P = 0.10$) to have a lower acetate-to-propionate ratio compared with cows fed silages stored for 30 d (30 d = 3.75 vs. 90 d = 3.34). Sodium benzoate increased ($P = 0.01$) plasma glucose from 63.6 mg/dL in the control treatment to 65.2 mg/dL in the benzoate treatment (Figure 1). The PUN was 22.6 mg/dL on average (SEM = 0.42) and had no statistical significance for treatment effect or interactions.

DISCUSSION

Grain silage conservation requires adequate moisture content to allow microbial growth and activity (Pahlow et al., 2003). Despite the wide range of moisture contents that result in well-fermented silages, the data in the literature suggest that 35% moisture is the optimal target to enhance fermentation, increase proteolysis, and improve starch degradability (Neuhaus and Totusek, 1971; Barol et al., 1986; Gomes et al., 2018). Based on these findings, we reconstituted sorghum grains with the aim of reaching 35% moisture. Although there was no difference between treatments, the average values of pH, lactic acid, and acetic acid were in agreement with the values of high-quality moisture grain silages in the literature (Morais et al., 2017).

The ethanol concentration was low in treated silages due to the direct effect of benzoic acid on yeast metabolism, which is the main microorganism responsible for ethanol production in silages (Pahlow et al., 2003). Although other weak acids, such as acetic, propionic, and butyric acids, can control yeasts in acidic conditions (Moon, 1983), these compounds were not altered when silages were treated with sodium benzoate. These findings support the hypothesis that benzoic acid was the compound responsible for the reduction in ethanol concentration. The formation of the ethyl esters of lactate and acetate, which depend on ethanol concentration, was also reduced because of the low concentration of ethanol in treated silages (Weiss, 2017). The trend of sodium benzoate reducing 2,3-butanediol in treated silages might be related to the inhibition of enterobacteria (Nishino and Shinde, 2007). Additionally, enterobacteria have been associated with proteolysis (McDonald et al., 1991). It has been suggested that so-

Table 3. Performance of dairy cows fed reconstituted sorghum grain silage stored for 30 or 90 d without (CON) or with (BEN) addition of sodium benzoate

	30 d		90 d			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	SL	SB	SL × SB
DMI, kg/d	23.4	23.6	23.9	22.8	0.58	0.84	0.31	0.40
DOMI,[2] kg/d	13.5	13.5	13.9	13.2	0.34	0.75	0.22	0.23
N intake, kg/d	0.647	0.634	0.647	0.606	0.0172	0.45	0.44	0.76
Milk, kg/d	29.4	30.6	31.4	31.0	1.25	0.10	0.55	0.27
ECM, kg/d	28.9	29.5	30.8	30.0	1.02	0.07	0.99	0.27
Milk components								
Fat, %	3.59	3.59	3.56	3.56	0.141	0.63	0.57	0.55
Fat, kg/d	1.012	1.057	1.128	1.096	0.0407	0.01	0.81	0.21
Protein, %	3.23	3.23	3.22	3.20	0.017	0.71	0.68	0.60
Protein, kg/d	0.956	0.960	1.029	0.973	0.0730	0.04	0.22	0.16
Lactose, %	4.63	4.59	4.61	4.61	0.045	0.70	0.20	0.28
Lactose, kg/d	1.399	1.389	1.495	1.426	0.0644	0.05	0.40	0.26
MUN, mg/dL	14.6	14.7	13.9	13.7	0.563	0.07	0.91	0.83
MUN, g/d	43.0	43.7	43.3	42.1	2.47	0.86	0.70	0.86
Milk/DMI	1.26	1.28	1.30	1.35	0.456	0.10	0.19	0.67
ECM/DOMI	2.13	2.19	2.23	2.30	0.094	0.10	0.29	0.77
Milk N/N intake, %	23.9	24.2	25.8	25.1	0.89	0.07	0.77	0.49

[1]Probabilities for the effects of storage length (SL), sodium benzoate (SB), and the interaction between storage length and sodium benzoate (SL × SB). Significant differences when $P \leq 0.05$, and trends when $P > 0.05$ and ≤ 0.10.

[2]Digestible organic matter intake.

Table 4. Digestibility of nutrients and feeding behavior of dairy cows fed reconstituted sorghum grain silage stored for 30 or 90 d without (CON) or with (BEN) addition of sodium benzoate

	30 d		90 d			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	SL	SB	SL × SB
NDF intake, kg/d	8.3	8.3	8.3	7.8	0.21	0.13	0.14	0.17
NDF intake, % of BW	1.25	1.25	1.25	1.19	0.03	0.17	0.17	0.18
Digestibility, %								
DM	57.9	57.8	58.8	59.2	0.72	0.10	0.86	0.69
OM	60.7	60.6	61.7	61.8	0.78	0.16	0.98	0.87
NDF	28.2	28.9	30.5	29.1	1.74	0.46	0.84	0.53
CP	53.6	54.4	57.1	57.0	2.12	0.10	0.83	0.81
Starch	87.0	86.8	89.2	89.3	0.12	0.04	0.95	0.89
Fecal starch, %	8.9	9.7	8.0	7.9	0.72	0.06	0.62	0.50
Chewing behavior, min/d								
Ingestion	225	218	213	229	13.8	0.96	0.65	0.26
Rumination	516	516	502	499	19.6	0.47	0.76	0.90
Chewing	742	727	714	728	28.7	0.53	0.98	0.47
Particle sorting, % as fed								
>19 mm	100	96	99	102	3.5	0.49	0.93	0.29
8–19 mm	98	98	99	99	0.76	0.27	0.94	0.44
<8 mm	101	101	101	100	0.32	0.20	0.30	0.23

[1]Probabilities for the effects of storage length (SL), sodium benzoate (SB) and the interaction between storage length and sodium benzoate (SL × SB). Significant differences when $P \leq 0.05$, and trends when $P > 0.05$ and ≤ 0.10.

dium benzoate can mitigate proteolysis in grain silages (Da Silva et al., 2015). However, protein solubility and ammonia nitrogen content, which indicate proteolysis, were not altered by sodium benzoate in this trial ($P > 0.10$).

Plant and bacterial enzymes mediate approximately 90% of the proteolysis in corn grain silages (Junges et al., 2017). When proteolysis is desirable, conditions that allow plant and bacterial proteolytic enzymes to function must be attempted. Moisture, processing, and length of storage have been shown to be the most important factors that increase the breakdown of the protein matrix, increasing starch and protein digestibility in grain silages (Benton et al., 2005; Hoffman et al., 2011). The degree of proteolysis in grain silages is highly correlated with in vitro starch degradability (Ferraretto et al., 2014). In the current trial, the increased proteolytic activity of silages stored for 90 d, compared with that of silages stored for 30 d, possibly provided ruminal microbes easy access to starch granules, resulting in faster degradation of starch, as indicated by the greater rate of gas production in vitro. We expected that sodium benzoate would slow the rate of gas production from treated silages due to the possible inhibition of proteolysis, but this result did not occur. Thus, based on the results of this work, we can say that at the concentration used (0.2% as-fed material), sodium benzoate likely did not affect the microbial metabolic pathways responsible for protein degradation.

In acidic conditions, some species of *Lactobacillus* can use lactate, enabling the formation of acetate and 1,2-propanediol (Oude Elferink et al., 2001). However, only 1,2-propanediol was increased with prolonged storage in this trial. Considering that each mole of lactate is metabolized to equimolar amounts of acetate and 1,2-propanediol, the 109-mg difference in 1,2-propanediol should result in similar production of acetic acid, which is too small to affect the final concentration of acetic acid. Moreover, 1,2-propanediol can be metabolized into 1-propanol and propionic acid by *Lactobacillus diolivorans* (Krooneman et al., 2002), biologically supporting the tendency of silages stored for 90 d to have high concentrations of these end fermentation products.

Ensiling high-moisture grains has been shown to increase starch and protein digestibility (Wilkerson et al., 1997) as a result of the breakdown of the protein matrix, which is an important physiochemical barrier to starch digestion in ruminants (Owens et al., 1986). In sorghum, proteins are distributed in the endosperm (75%), germ (22%), and pericarp (3%; Bean et al., 2016). As mentioned above, certain main conditions enable proteolysis throughout ensiling; however, it is important to emphasize that when proteolysis is desired, physical barriers should not limit bacterial access to the endosperm. Compiled data from experiments published between 2012 and 2016 showed 5 to 10% increases in the in vitro starch degradability of corn silage within 45 d of ensiling and similar incremental increases after 45 and 120 d of ensiling (Kung et al., 2018). In reconstituted grain silages, starch degradability seems to increase in smaller increments than it does in corn

silage (Gomes et al., 2018), and storage longer than 2 mo might be needed to optimize starch digestibility (Da Silva et al., 2019).

In the current trial, the 60-d difference in storage period tended to allowed cows produce 1.2 kg/d more milk without changing DMI. These responses indicate greater feed efficiency for the 90-d compared with the 30-d treatment. The greater milk production of cows fed silages stored for 90 d is partially explained by the increase in starch and protein digestibility. Feeding diets with highly digestible starch often improves feed efficiency, by either increasing milk yield with similar DMI (Oba and Allen, 2003; Arcari et al., 2016) or decreasing DMI and maintaining milk yield (McCaffree and Merrill, 1968; Ferraretto et al., 2013).

The energy content of 1.2 kg of milk, considering the average percentage of fat (3.57%), protein (3.22%), and lactose (4.61%), was calculated as 0.826 Mcal. The difference in starch (2.35%) and protein (3.05%) digestibility contributes with an additional 178 and 120 g of digested starch and protein, respectively. Applying the coefficient efficiency of 0.82 from digestible energy (DE) to ME (NRC, 2001) and 0.64 from ME to milk energy production (Moe and Tyrrell, 1972), we estimated that the increases in starch and protein digestibility explained 47.5 and 41.1%, respectively, of the difference in milk yield based on energy partition, which correspond to 88.6% of the total. The remaining 11.4% to complete the 100% might be a consequence of microbial protein synthesis, although we did not measure any variable to estimate the contribution of this source.

The MUN tended to reduce, by 0.85 mg/dL, with the 90-d treatment compared with 30 d, even though diet CP was similar. Nousiainen et al. (2004) reported that diet CP was a more accurate predictor of MUN than was the CP/ME ratio. Conversely, Fadul-Pacheco et al. (2015) found a low correlation between CP and MUN, suggesting that an improved understanding of other herd characteristics may be needed to explain differences in MUN. Using the Nousiainen equation, we estimated the MUN in the present study to be 14.4 mg/dL, which underestimated the MUN for diets with silages stored for 30 d and overestimated the MUN for diets with silages stored for 90 d. This finding suggests increased ruminal use of ammonia nitrogen when cows were fed a grain silage with an increased rate of starch degradation. Because the concentration of ammonia nitrogen in the rumen was higher in silages stored for 90 d, due to increased protein solubility and deamination, the large supply of energy available to the ruminal microbes might have increased AA utilization without a transient ammonia nitrogen pool (Hristov et al., 2005). The PUN, which is highly correlated with MUN (Baker et al., 1995), did not differ between the treatments. This inconsistency between PUN and MUN concentration is probably due to the difference in milk production levels of the treatments, which diluted the MUN.

The high starch digestibility of the diets containing grain silage stored for 90 d reduced the MUN concen-

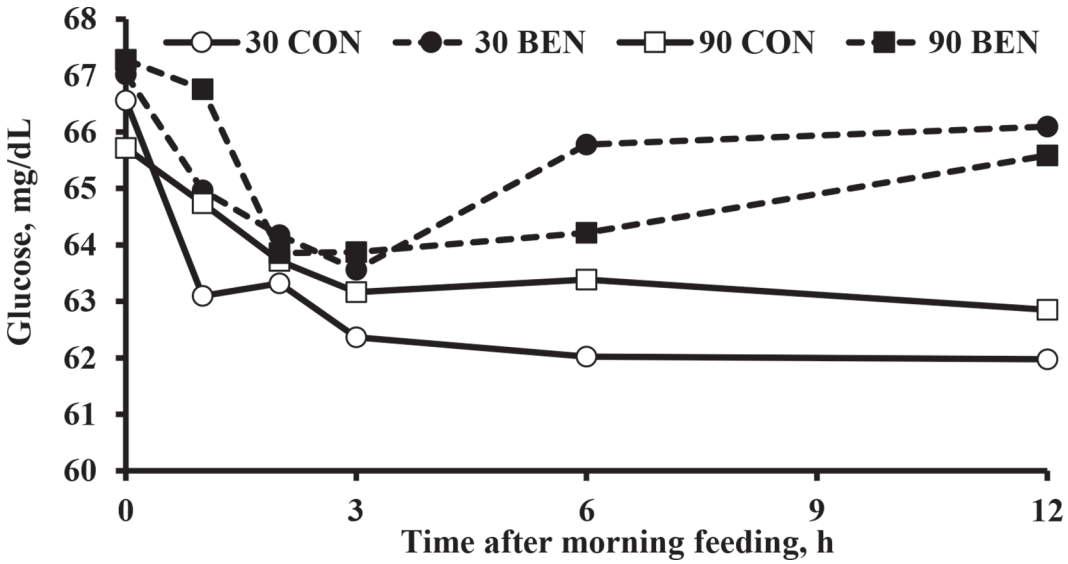

Figure 1. Plasma glucose concentration in dairy cows fed reconstituted sorghum grain silages stored for 30 or 90 d without (CON) or with (BEN) addition of sodium benzoate. *P*-values: storage length = 0.56, sodium benzoate = 0.01, interaction of storage length and sodium benzoate = 0.51, hour <0.01, interaction of storage length and hour = 0.83, sodium benzoate hour = 0.58, and among storage length, sodium benzoate, and hour = 0.90. SEM = 0.847. Significant differences when $P \leq 0.05$, and trends when $P > 0.05$ and ≤ 0.10.

Table 5. Ruminal fermentation profile of cannulated dairy cows fed reconstituted sorghum grain silage stored for 30 or 90 d without (CON) or with (BEN) addition of sodium benzoate (data from 4 cows arranged in a 4 × 4 Latin square)

Item	30 d		90 d		SEM	*P*-value[1]					
	CON	BEN	CON	BEN		SL	SB	SL × SB	H	SL × H	SB × H	SL × SB × H
pH	6.42	6.51	6.55	6.57	0.056	0.12	0.32	0.46	<0.01	0.71	0.72	0.16
Ammonia nitrogen, mg/dL	9.52	8.40	11.15	10.26	0.97	0.04	0.22	0.88	0.83	0.55	0.91	0.94
VFA proportion, mol/100 mol												
Acetate	64.6	65.8	64.8	64.4	0.68	0.73	0.16	0.54	0.01	0.92	0.58	0.88
Propionate	17.6	17.3	19.3	19.3	0.96	0.14	0.87	0.49	<0.01	0.85	0.67	0.40
Butyrate	11.9	11.0	10.9	11.0	0.42	0.27	0.36	0.27	0.17	0.12	0.47	0.81
Isobutyrate	1.2	1.3	1.2	1.2	0.07	0.59	0.58	0.33	0.01	0.29	0.19	0.81
Valerate	1.4	1.4	1.3	1.4	0.06	0.25	0.22	0.92	0.07	0.17	0.97	0.44
Isovalerate	2.7	2.7	2.5	2.6	0.12	0.34	0.81	0.89	<0.01	0.56	0.13	0.97
Acetate-to-propionate ratio	3.67	3.82	3.35	3.33	0.213	0.10	0.58	0.40	0.01	0.95	0.63	0.67
Total VFA, mmol/L	120.9	122.1	123.8	129.3	3.24	0.44	0.13	0.94	0.03	0.92	0.37	0.13

[1]Probabilities for the effects of storage length (SL), sodium benzoate (SB), the interaction between storage length and sodium benzoate (SL × SB), hour (H), the interaction between storage length and hour (SL × H), the interaction between sodium benzoate and hour (SB × H), and the interactions among storage length, sodium benzoate, and hour (SL × SB × H). Significant differences when $P \leq 0.05$, and trends when $P > 0.05$ and ≤ 0.10.

tration. However, daily excretion of MUN was not altered (43.02 g/d). Hence, the reduction in MUN can be mathematically explained as a consequence of dilution. On the other hand, the daily excretion of milk protein of cows fed silage stored for 90 d was 43 g higher than that of cows fed silage stored for 30 d. The supply of AA to support this difference in protein yield probably came from rumen microbes and sorghum proteins, which had greater digestibility as a result of extensive proteolysis. In the current study, cows that received diets with highly digestible RSGS were more efficient at converting nitrogen from the diet to milk nitrogen. With the same nitrogen intake, as in this trial, the only way to increase milk nitrogen efficiency is by retaining more nitrogen as milk components.

The ruminal fermentation profile was not changed by lengthening silage storage (Table 5). Oba and Allen (2003) fed high-producing dairy cows dry corn or high-moisture corn and detected an increase in total VFA concentration with the high-moisture corn diet compared with the dry corn diet, without a shift in VFA proportions. Changes in ruminal fermentation patterns were observed when the NDF/NFC ratio was drastically altered by replacing forage for concentrate (Russell, 1998; Sutton et al., 2003). Although starch digestibility increased with the length of storage, the forage-to-concentrate ratio was similar in this trial.

Sodium benzoate increased the concentration of plasma glucose. Intravenous injection of sodium benzoate in sheep has been associated with the pancreatic endocrine system, inducing insulin and glucagon secretion (Mineo et al., 1995). Phillips et al. (1969) found that injection of short-chain FA and glucagon in normal and depancreatized sheep resulted in acid-induced hyperglycemia. Mineo et al. (1995) suggested that a similar mechanism is involved in the change to plasma glucose following benzoic acid administration. Benzoic acid has also been described as a stimulant of pancreatic amylase secretion in sheep (Katoh and Yajima, 1989). Thus, further studies are warranted to confirm the effects of sodium benzoate on glucose concentration in dairy cows.

CONCLUSIONS

Increasing the storage length of reconstituted sorghum grain silage from 30 to 90 d increased starch digestibility, milk yield, feed efficiency, and milk nitrogen efficiency. Sodium benzoate reduced silage ethanol concentration and did not alter the performance of dairy cows. Further studies are warranted to determine the effect of sodium benzoate on the glucose metabolism of dairy cows.

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