Improvement of feed intake, digestibility, plasma metabolites, and lactation performance of dairy cows fed mixed silage of sugar beet pulp and rice straw inoculated with lactic acid bacteria

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ABSTRACT

A study was conducted to investigate the inclusion effects of sugar beet pulp and rice straw mixture silage with inoculation (BRMS), in place of whole-plant corn silage (CS), on the dry matter intake, total-tract nutrient digestibility, plasma metabolites, rumen fermentation, and lactation performance in high-production dairy cows. Sixteen multiparous Holstein cows (body weight, 622 ± 35 kg; days in milk, 90 ± 11 d; mean $\pm$ standard deviation) were used in our experiments; the experiments were based on a repeated 4×4 Latin square design for 21 d, and each experimental period consisted of 14 d of adaptation, followed by 7 d of data collection. The 4 dietary treatments used were (dry matter basis): (1) 0% BRMS and 28.6% CS (0BRMS); (2) 4.3% BRMS and 24.3% CS (15BRMS); (3) 8.60% BRMS and 20.0% CS (30BRMS); and (4) 12.9% BRMS and 15.7% CS (45BRMS). The increasing inclusion of dietary BRMS was observed to linearly increase the total volatile fatty acids and the propionate concentration. The dry matter intake and digestibility values of neutral detergent fiber and acid detergent fiber increased linearly as the percentage of BRMS increased up to 45%. Milk yield linearly increased with the increase in the content of BRMS (39.0, 39.8, 40.9, and 40.3 kg/d for 0BRMS, 15BRMS, 30BRMS, and 45BRMS, respectively). The increasing inclusion of dietary BRMS induced a decrease in the ammonia nitrogen and milk urea nitrogen concentration, leading to a linear increase in milk protein production (1.15, 1.26, 1.35, and 1.27 kg/d for 0BRMS, 15BRMS, 30BRMS, and 45BRMS,

respectively). In conclusion, the diets with the replacement of CS with BRMS up to 45% were beneficial to the production performance of high-production dairy cows, indicating that this method may be an appropriate use of sugar beet pulp and rice straw.

Key words: sugar beet pulp, mixture silage, inoculation, milk yield, milk protein

INTRODUCTION

High quality forage is critical to maintain rumen function, milk yield, and the health of high-producing dairy cows; dietary inclusion of forages with highly digestible NDF [whole-plant corn silage (CS) and alfalfa hay] have the potential to promote feed intake and increase milk yield (Wang et al., 2014; Ferraretto et al., 2015). However, both forages require considerable area of land and water and are difficult to achieve in unfavorable environments, which are encountered in several regions. Additionally, the excessive usage of dietary CS with a high starch content not only causes a risk of rumen acidosis (Kmicikewycz and Heinrichs, 2015), but also creates competition for human-edible feed (Vojvodić et al., 2016).

To compensate for the insufficient amount of high quality forage, feeding soybean husk, sugar beet pulp (SBP), apple pomace, and citrus pulp are viable options to improve the sustainability of dairy production systems by reducing the risk of rumen acidosis and decreasing the dietary proportion of human-edible products (Vojvodić et al., 2016; Zhao et al., 2017), and minimize the use of agricultural land and water for feed production (NRC, 2001). The inclusion of dietary byproducts with highly digestible NDF can replace CS with high digestible starch (Naderi et al., 2016; Heydari et al., 2021), and Miron et al. (2010) pointed out the necessity of increasing the converting efficiency from

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feed nutrients to animal products in high-production dairy cows.

Sugar beet pulp, which is a byproduct of sugar beet processing, is a common ingredient in the diet of dairy cows, and has a high NDF (Naderi et al., 2016) and pectic content (NRC, 2001). Fresh SBP has a high annual output (more than 3 million tons) in Heilongjiang province; therefore, it is an inexpensive fiber source that can be of interest to local farmers. Additionally, the SBP has high degradable NDF, pectic, and hemicellulose (galactan and arabinan) contents, and Ferraretto et al. (2015) reported that the DMI and milk of dairy cows fed CS with high NDF digestibility were higher than those fed CS with high starch digestibility, which indicated that the energy supply in the form of NDF is more efficient than that supplied in the form of starch. Miron et al. (2010) and Vojvodić et al. (2016) demonstrated that the dietary inclusion of nonforage fiber sources with high degradable fiber improved intake, health, and productivity. Several studies have shown that the inclusion of dietary SBP as nonforage fiber sources have positive effects on the production performance of dairy cows; Naderi et al. (2016) demonstrated that the use of SBP decreased the rumen ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration, corresponding to an increase in the milk yield and protein percentage, and Kahyani et al. (2019a) reported that the feed intake and milk protein yield of dairy cows tended to increase with the inclusion of SBP in the diet, which may be related to particle size, palatability, nutrient digestibility, and nitrogen utilization efficiency.

Fresh SBP is not easily ensiled owing to its high moisture content, and it is usually processed via drying. Drying is an energy- and cost-intensive procedure due to heat loss, and it causes air pollution-related problems; therefore, ensiling is considered an effective method to maintain the original nutritional value and extend the storage time of products. The inclusion of rice straw in the silage can not only reduce the moisture content to ensure the success of silage, but also can make use of large resources of rice straw (0.23 billion/yr in China), effectively reducing environmental pollution induced by burning and discarding. Ensiling SBP and rice straw theoretically compensates for the insufficiency of physically effective NDF (**peNDF**) because rice straw is rich in lignocellulose and increases the fermentable substrate content, and because SBP has a high soluble fiber content.

Lactic acid bacteria (**LAB**) have been used as an additive to enhance the fermentation quality and the digestibility of straw silage (Guo et al., 2020), and ensiling high moisture amaranth and rice straw with inoculation (**LAB**) enhances the silage fermentation

quality (Mu et al., 2020), and the silage with inoculation (**LAB**) leads to a rapid reduction of the pH and acetic acid content (Oliveira et al., 2017).

It was hypothesized that the replacement of CS with sugar beet pulp and rice straw mixture silage (**BRMS**) would increase feed intake and milk yield of dairy cows. In this study, we developed a novel silage and investigated the inclusion effect of increasing the dietary concentration of **BRMS** at the expense of CS on the feed intake, total-tract nutrient digestibility, rumen fermentation, plasma metabolites, and lactation performance. The objective was to develop a high-quality forage for high-production dairy cows, which decreases the cost of feed and reduces environmental pollution.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All experiments were conducted according to the principles and guidelines approved by the Animal Care and Use Committee of the Northeast Agriculture University. Sixteen multiparous Holstein cows (BW, 622 ± 35 kg; DIM, 90 ± 11 d; parity 2 ± 1.2 ; mean $\pm$ SD) were used in these experiments; the experiments were based on a repeated 4×4 Latin square design of 21 d (14 d of adaptation and 7 d of data collection). The dietary ingredients and chemical compositions are presented in Table 1, and Diamond V XP [the content of dried soy wine yeast culture (IFN7-05-520), 100%; Diamond V Mills] and MetaSmart [$\text{CH}_3\text{S}-(\text{CH}_2)_2\text{-CH}(\text{OH})\text{-COO-CH}(\text{CH}_3)_2$ and CAS-Nr (7631-86-9): $10\text{SiO}_2(\text{H}_2\text{O})$, less than 57%; Adisseo] were used in the diet. The diet was formulated to meet the requirements of the dairy cows regarding protein, energy, minerals, and vitamins based on the Cornell-Penn-Miner model, and the treatment diets contained similar ingredients and forages, but different portions of CS and **BRMS** (replacing 0%, 15%, 30%, and 45% CS with **BRMS**, respectively, DM basis), and the oat hay needed to be chopped with a fodder chopper before the diet was sent to the TMR machine. Cows were housed in individual stalls, and clean wood shavings and sand were used for bedding and were refreshed daily. Cows were fed the TMR twice daily with ad libitum access to feed at 0600 and 1700 h; the animals were allowed a 5% feed refusal, and the refusal was removed and weighed every day. The cows had free access to drinking water.

Sample Collection, Measurements, and Analyses

The CS (Wanghe No. 8) was provided from Jiusan Farm in Heilongjiang and was ensiled for 60 d in a

silage pit; the harvest time of the CS lasted from September 9 to September 10, 2020. The CS samples were collected from multiple (8) spots of the received material and in triplicate from each spot. The SBP was obtained from Nanhua Sugar Trading Co. Ltd. Jiusan Farm in Heilongjiang, and the samples were collected from multiple spots (5 samples), and in triplicate from each spot. The rice straw was provided by Jiusan Farm and was chopped to approximately 2 to 3 cm in length using a fodder chopper. The mixture (BRM) was composed by the SBP sample and rice straw at a ratio of 9:1 (fresh material) manually, and it was sprayed with inoculants (5×10^9 LAB/g) of fresh forage at 1

$\times 10^{11}$ cfu/t according to the recommended dose. Ten kilograms of each subsample was sampled and packed into laboratory-scale silos with ventilation (10 kg, 46 cm $\times$ 65 cm $\times$ 0.2 mm); then the bags were sealed with a vacuum sealer (BH 950; Matsushita) and ensiled for 60 d (BRMS). The LAB inoculant (*Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC BAA-365; *Lactobacillus acidophilus* ACCC10637, 1:1) was provided by Xin-laiwang Biotechnology Co. Ltd. The BRM and BRMS were sampled for chemical analysis.

The samples, including BRM, BRMS, CS, and TMR, were dried at 60°C in a forced-air oven for 48 h and ground using a Wiley mill through a 1-mm screen

Table 1. Ingredients and chemical composition [mean (SD)] of the experimental diets

Item	Diet ¹			
	Control	15BRMS	30BRMS	45BRMS
Ingredient (% of DM)				
BRMS	—	4.30	8.57	12.9
Corn silage	28.6	24.3	20.00	15.7
Corn	22.5	22.5	22.5	22.6
Alfalfa hay	11.3	11.3	11.3	11.3
Oat hay	6.14	6.14	6.14	6.14
Soybean meal	1.59	1.59	1.59	1.59
Rapeseed meal	9.40	9.40	9.40	9.40
Wheat bran	9.27	9.27	9.27	9.27
Cottonseed meal	7.44	7.44	7.44	7.44
2% Premix ²	1.00	1.00	1.00	1.00
Calcium carbonate	0.79	0.79	0.79	0.79
Sodium bicarbonate	0.84	0.84	0.84	0.84
Diamond V XP ³ (yeast culture)	0.38	0.38	0.38	0.38
Calcium phosphate dihydrate	0.42	0.42	0.42	0.42
Salt/NaCl	0.21	0.21	0.21	0.21
MetaSmart ⁴ (rumen-protected Met)	0.17	0.17	0.17	0.17
Chemical composition (% of DM)				
CP	17.0 (0.37)	17.2 (0.22)	17.4 (0.23)	17.6 (0.20)
SCP ⁵ (% of CP)	31.7 (0.35)	31.6 (0.44)	31.7 (0.36)	30.9 (0.35)
NDF	31.7 (0.44)	32.3 (0.57)	32.8 (0.43)	33.4 (0.12)
peNDF ⁶ (% of DM)	23.1 (0.56)	22.4 (0.78)	21.7 (0.55)	21.0 (0.64)
ADF	19.4 (0.18)	19.5 (0.41)	19.6 (0.34)	19.7 (0.34)
ADL	3.81 (0.14)	3.91 (0.12)	4.0 (0.14)	4.10 (0.19)
NFC ⁷	42.0 (0.36)	41.1 (0.36)	40.4 (0.39)	39.6 (0.20)
Ether extract	3.59 (0.18)	3.56 (0.28)	3.5 (0.13)	3.48 (0.22)
Starch	29.3 (0.35)	28.1 (0.44)	27.0 (0.32)	25.8 (0.21)
Soluble fiber	5.48 (0.44)	6.12 (0.30)	6.75 (0.23)	7.39 (0.15)

¹15BRMS, 30BRMS, and 45BRMS indicate the 15%, 30%, and 45% replacement of corn silage with sugar beet pulp and rice straw mixture silage (BRMS), respectively.

²Premix was composed of vitamin A, vitamin D₃, vitamin E, manganese sulfate, zinc sulfate, magnesium oxide, limestone, and sodium chloride, and the chemical composition of the premix contained (DM basis) 99.17% ash, 14.25% Ca, 5.40% P, 4.93% Mg, 0.05% K, 10.64% Na, 2.95% Cl, 0.37% S, 12 mg/kg Co, 500 mg/kg Cu, 4,858 mg/kg Fe, 25 mg/kg I, 800 mg/kg Mn, 10 mg/kg Se, 1,800 mg/kg Zn, 180,000 IU/kg vitamin A, 55,000 IU/kg vitamin D, and 1,500 IU/kg vitamin E (Diamond V Mills).

³Diamond V XP, the content of dried soy wine yeast culture (IFN7-05-520) is 100%, and guaranteed values are CP $\geq$ 15%, ether extract $\geq$ 0.5%, CF $\leq$ 32%, ash $\leq$ 12%, water $\leq$ 10%, mannan $\geq$ 2% (Diamond V Mills).

⁴MetaSmart is composed of CH₃S-(CH₂)₂-CH(OH)-COO-CH-(CH₃)₂ and CAS-Nr [7631-86-9]: 10SiO₂(H₂O), and the guaranteed value of 2-hydroxy-4-methylthiobutyrate isopropyl is not lower than 57% (Adisseo).

⁵SCP = soluble crude protein.

⁶peNDF = physically effective NDF determined as the NDF content of TMR multiplied by physically effective fiber >1.18 .

⁷NFC = 100 - % NDF - % (CP - neutral detergent insoluble crude protein) - % ether extract - % ash.

(FZ102, Taisite Instrument Co. Ltd.). The BRM samples were collected by multipoint sampling, and the nutrition compositions of BRM were analyzed. The BRMS, CS, and TMR samples were collected weekly, and the nutrition compositions were analyzed. The DM, ether extract, and CP contents were analyzed according to the procedures 934.01, 920.35, and 976.05, respectively, of AOAC (1990). The NDF and ADF contents were sequentially analyzed using the Ankom A200 fiber analyzer (Ankom Technology), and the NDF content was determined according to the method by Van Soest et al. (1991) using heat-stable α -amylase and sodium sulfite, and the ADF content was determined according to the 954.01 procedure of AOAC (1990). The starch content was analyzed using the anthrone shade selection method (Guo et al., 2007). The neutral detergent insoluble crude protein (**NDICP**) and acid detergent insoluble CP contents were measured by analyzing the CP content in NDF and ADF, respectively. The ADL content was determined using the methods of Licitra et al. (1996). The soluble CP content was measured using the Folin-phenol method (Licitra et al., 1996), and the NPN content was calculated as the difference between the soluble protein and the protein precipitated in trichloroacetic acid (Licitra et al., 1996). The water-soluble carbohydrate (**WSC**) content was determined using the sulfuric acid-anthrone method (Sun et al., 2016). The physically effective fiber (**pef**) and peNDF contents were determined using the method by Yang and Beauchemin (2006). Particle size was measured in triplicate using the Penn State Particle Separator equipped with 3 sieves and a bottom pan. The formulas used for the pef and peNDF were $\text{pef}_{1.18} = \text{DM } 19 \text{ mm (the proportion of particles retained)} + \text{DM } 8 \text{ mm (the proportion of particles retained)} + \text{DM } 1.18 \text{ mm (the proportion of particles retained)}/100$ and $\text{peNDF} = \text{pef} \times \text{NDF}\%$, respectively.

The quality of the silage was evaluated using the V-score rating system (Gao et al., 2016). The pH value was determined using a pH meter (Sartorius, PB-10). The lactic acid concentration was determined using HPLC, and the concentrations of VFA were measured using a Shimadzu GC-2010 gas chromatograph (Jiang et al., 2020; Agilent capillary column (30.0 m $\times$ 320 μm $\times$ 0.5 μm ; Agilent Technologies Inc.). The loading conditions used were that the temperature of the inlet and the detector was 220°C; heating temperature of the column oven: the initial temperature was 120°C, which was maintained for 3 min, then was increased to 180°C at 10°C/min; the carrier gas used was high-purity nitrogen; the inlet pressure was maintained at 90 kPa; the flow rate of hydrogen used was 40 mL/min; the air flow rate was 400 mL/min; and the makeup flow rate was 45 mL/min. The ruminal $\text{NH}_3\text{-N}$ concentration was

determined using the colorimetric phenol-hypochlorite method described by Broderick and Kang (1980). The concentration values of mycotoxins (aflatoxin B₁, zearalenone, deoxynivalenol, and ochratoxin) in the CS and BRMS were determined by the Inspection and Quarantine Technology Center of Heilongjiang Entry-Exit Inspection and Quarantine Bureau, according to the GB/T 30955 (Standardization Administration, 2014), GB/T 28716 (Standardization Administration, 2012), GB/T 30956 (Standardization Administration, 2014), and GB/T 30957 (Standardization Administration, 2014) methods, respectively.

Intake and Digestibility

The TMR offered and refused was measured daily for each cow, and the nutrient compositions (DM, CP, and NDF) of TMR and the refusals were determined. The DMI value for each cow was calculated, and refusals from individual cows were used to calculate the nutrient (DM, CP, and NDF) intake. On the last 3 d of each experimental period, approximately 500 g of the fecal samples were collected from the rectum at 0600 and 1800 h and composited for each individual cow. The apparent total-tract digestibility values of nutrients were determined using acid-insoluble ash as an internal marker (Lee and Hristov, 2013).

Rumen Sampling and Analyses

On d 19, 20, and 21 of each period, ruminal fluid samples were collected using an oral stomach tube approximately 3 h after the morning feed delivery (Wang et al., 2014). We threw away the first 100 to 200 mL of fluid collected through the stomach tube to reduce the chance that stomach-tube rumen samples were contaminated with saliva before ruminal fluid samples were collected. Ruminal digesta samples were squeezed in a 4-layer cheesecloth to obtain a ruminal fluid sample (250 mL). The rumen fluid was centrifuged at $1,500 \times g$ for 15 min, and the supernatant was collected and frozen for determination of the concentration of $\text{NH}_3\text{-N}$ and VFA. The methods used for the $\text{NH}_3\text{-N}$ and VFA assays were the same as the methods used for the analyses of fresh samples.

Plasma Sampling and Analysis

On d 18 of each period, blood samples (10 mL) were collected via the coccygeal vein with an anticoagulant at approximately 3 h after the morning feed. Blood samples were placed on ice immediately after collection and centrifuged at $3,000 \times g$ for 15 min, and the plasma was separated and frozen to -20°C until analysis. Com-

mercial kits (Nanjing Jiancheng Bioengineering Institute) were used to determine the plasma concentration values of glucose, triglyceride, total cholesterol, BHB, urea nitrogen (UN), and total protein (TP), according to the manufacturer's instructions.

Milk Yield and Components

The dairy cows were milked 3 times a day at 0700, 1500, and 2300 h, and the milk yield was recorded at each milking. On d 15, 16, and 17 of each period, the milk samples (50 mL) were collected in the morning, afternoon, and evening, and mixed well at a ratio of 4:3:3 (based on the production), then preserved with potassium dichromate and stored at 4°C. The milk samples were sent to Nestlé DFI to determine the concentration of fat, milk, and lactose using an infrared milk composition analyzer (MilkoScan 605, Foss Electric). The concentration of MUN was determined using an enzymatic assay (Wilson et al., 1998). The 3.5% FCM and ECM were calculated according to the guidelines from NRC (2001): $FCM = 0.432 \times \text{milk yield} + 16.23 \times \text{fat yield}$; $ECM = \text{milk production} \times (0.383 \times \% \text{ fat} + 0.242 \times \% \text{ protein} + 0.7832)/3.1183$.

Data Processing

The data were analyzed using a Latin square design and the MIXED procedure of SAS (9.3.1, SAS Institute Inc.), and the differences among the treatments were assessed using LSMEANS with the PDIF procedure in SAS. The following model was used for the analysis as a replicated 4×4 Latin square design: $Y_{ijkm} = \mu + S_i + C(S)_{ij} + P_k + T_m + E_{ijkm}$, where Y_{ijkm} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square I , $C(S)_{ij}$ is the random effect of cow j within square I , P_k is the fixed effect of period k , T_m is the fixed effect of treatment m , and E_{ijkm} is the residual error. Differences were considered statistically significant at $P \leq 0.05$, and highly significant values were defined as $P < 0.01$, and trends were reported at $0.05 < P \leq 0.10$. Orthogonal polynomial contrasts (linear, quadratic, and cubic) were used to examine the treatment effects on the response variables.

RESULTS

Chemical Composition of the Nutritional Ingredients of BRMS

The chemical composition of SBP and rice straw are presented in Table 2. The CP and NDF contents of fresh SBP were 14.8% DM and 51% DM, respectively,

and the NDF content of rice straw was 75% DM, which are all higher than those obtained previously. The DM content of the BRM at a ratio of 9 to 1 was 300 g/kg, and the WSC content was 41.1 g/kg.

The chemical composition (BRM, BRMS, and CS) and silage quality (BRMS and CS) are presented in Table 3, and the contents of the different mycotoxins are presented in Table 4. The CP content of BRMS was higher than that of BRM, and increased by 5.78%. The DM and WSC contents of BRMS were lower than those of BRM, and decreased by 5.67% and 17.07%, respectively. The BRMS had a low starch content (3.2% DM) compared with that of CS (30.6% DM); therefore, the dietary starch content decreased by 1.2%, 2.3%, and 3.5%, respectively, with the increasing inclusion of BRMS, which rapidly reduced the risk of rumen acidosis caused by starch fermentation. The SBP had a higher NDICP content (6.81% DM, an indicator of RUP) compared with that of CS, which indicated that it had a high efficiency of protein utilization. The CS and BRMS were above 80, as evaluated using the V-score system, and the levels of aflatoxin B₁, zearalenone, deoxynivalenol, and ochratoxin A were all far below the prescribed limitation values, which indicated that the BRMS had high quality and safety.

Intake and Total-Tract Digestibility of Nutrients

The nutrient intake and total-tract digestibility of replacing CS with BRMS are listed in Table 5. The CP, NDF, and ADF intake linearly increased with the in-

Table 2. Chemical composition and quality evaluation of sugar beet pulp and rice straw (% of DM unless otherwise noted; mean $\pm$ SD)

Item ¹	Sugar beet pulp	Rice straw
DM (%)	23.0 $\pm$ 0.26	93.0 $\pm$ 0.35
CP	14.8 $\pm$ 0.24	5.4 $\pm$ 0.22
Soluble protein	6.37 $\pm$ 0.30	1.89 $\pm$ 0.10
NPN	5.92 $\pm$ 0.32	1.82 $\pm$ 0.21
ADICP	0.85 $\pm$ 0.04	0.33 $\pm$ 0.10
NDICP	6.81 $\pm$ 0.23	1.68 $\pm$ 0.20
ADF	23.0 $\pm$ 0.33	45.0 $\pm$ 1.96
NDF	51.1 $\pm$ 0.95	75.0 $\pm$ 0.37
peNDF	25.6 $\pm$ 0.34	75.0 $\pm$ 0.37
ADL	5.31 $\pm$ 0.23	10.5 $\pm$ 0.25
Ash	11.9 $\pm$ 0.40	10.5 $\pm$ 0.27
NFC	28.1 $\pm$ 0.3	7.17 $\pm$ 0.18
Ether extract	0.90 $\pm$ 0.08	—
Sugar	5.06 $\pm$ 0.19	2.30 $\pm$ 0.22
Starch	2.81 $\pm$ 0.22	1.29 $\pm$ 0.16
WSC	4.99 $\pm$ 0.03	2.17 $\pm$ 0.21

¹ADICP = acid detergent insoluble crude protein; NDICP = neutral detergent insoluble crude protein; peNDF = NDF $\times$ pef (physically effective fiber) > 1.18 (3 sieve) (Penn State Particle Separator); NFC = $100 - \% \text{ NDF} - \% (\text{CP} - \text{NDICP}) - \% \text{ ether extract} - \% \text{ ash}$; WSC = water-soluble carbohydrates.

Table 3. Chemical composition and quality evaluation of sugar beet pulp and rice straw mixture (BRM), sugar beet pulp and rice straw mixture silage (BRMS), and corn silage [% of DM unless otherwise noted; mean (SD)]

Item ¹	BRM	BRMS	Corn silage
DM (%)	30.0 (0.38)	28.7 (0.11)	28.5 (0.51)
CP	12.1 (0.24)	12.8 (0.91)	7.8 (0.22)
Soluble CP	5.6 (0.11)	5.7 (0.16)	4.6 (0.32)
NPN	5.2 (0.22)	5.3 (0.16)	4.6 (0.35)
ADICP	0.7 (0.02)	0.7 (0.05)	0.9 (0.11)
NDICP	5.3 (0.07)	5.3 (0.12)	1.2 (0.15)
ADF	29.8 (0.23)	28.9 (0.24)	27.5 (0.44)
NDF	58.5 (0.41)	57.7 (0.20)	45.3 (0.56)
peNDF	40.9 (0.33)	40.7 (0.15)	39.8 (1.22)
ADL	6.1 (0.31)	6.1 (0.25)	3.7 (0.11)
Ash	11.5 (0.21)	11.5 (0.07)	5.1 (0.14)
NFC	21.5 (0.28)	23.6 (0.32)	40.4 (1.05)
Starch	1.4 (0.09)	1.3 (0.19)	29.9 (0.85)
WSC	4.1 (0.26)	3.4 (0.26)	3.7 (0.37)
Fermentation quality			
pH	—	3.9 (0.25)	4.1 (0.15)
Lactic acid	—	4.3 (0.33)	3.8 (0.18)
Acetic acid	—	1.8 (0.09)	0.9 (0.07)
Propionic acid	—	0.2 (0.02)	0.1 (0.01)
Butyric acid	—	—	0.1 (0.01)
NH ₃ -N/total N	—	3.15 (0.15)	5.56 (0.22)
Score	—	>80	>80

¹ADICP, acid detergent insoluble crude protein; NDICP, neutral detergent insoluble crude protein; peNDF = NDF × pef (physically effective fiber) _{>1.18} (Penn State Particle Separator); NFC = 100 – % NDF – % (CP – NDICP) – % ether extract – % ash.

crease in BRMS to up to 45% ($P = 0.02$, $P < 0.01$, and $P = 0.02$, respectively), and they increased by 8.68%, 10.78%, and 6.73%, respectively. The peNDF linearly tended to decrease with the increasing replacement with BRMS up to 45% ($P = 0.07$). The DM digestibility linearly tended to increase as the BRMS increased to up to 45% ($P = 0.07$), and it quadratically increased ($P = 0.02$), and it increased by 2.52%. The CP digestibility linearly tended to increase as the dietary BRMS reached 45% ($P = 0.07$). The NDF and ADF digestibility values linearly increased as the BRMS increased up to 45% ($P < 0.01$ and $P = 0.02$, respectively), and increased to 54.5% and 46.6%, respectively.

Rumen Fermentation

The effects of replacing CS with BRMS on rumen fermentation are presented in Table 6. The total VFA

concentration in the rumen linearly increased to 101 mmol/L with the inclusion of dietary BRMS, increasing up to 45% ($P = 0.04$), and the propionate acid concentration increased to 23.9 mmol/L as the substitution of BRMS increased up to 45% ($P = 0.02$). The ruminal caproic concentration linearly tended to increase with the increasing replacement with BRMS up to 45% ($P = 0.08$). The ruminal NH₃-N concentration linearly decreased as the dietary BRMS reached 45% ($P = 0.01$), and decreased by 4.1%. The ratio of the concentrations of acetate and butyrate was not affected by increasing the content of dietary BRMS.

Analysis of Plasma Metabolites

The effects of replacing CS with BRMS on plasma metabolites are shown in Table 7. The plasma concentrations of glucose, triglyceride, and albumin/globulin were not affected by the diet ($P > 0.10$). The plasma concentration of TP linearly increased ($P = 0.01$) as the BRMS increased to up to 45%, and increased by 2.62%. The plasma concentration of BHB linearly decreased with the increasing replacement with BRMS up to 45% ($P = 0.02$), and plasma concentration of BHB decreased by 13.33%. The plasma concentration of UN linearly decreased as the replacement of BRMS increased up to 45% ($P = 0.02$), and plasma concentration of UN decreased by 5.49%.

Table 4. Concentration of different mycotoxins (µg/kg) of sugar beet pulp and rice straw mixture silage (BRMS) and corn silage

Item	BRMS	Corn silage	Range of calibration
Aflatoxin B ₁	<0.2	<0.2	<20
Zearalenone	<0.2	<0.2	<500
Deoxynivalenol	98.2	165	<5,000
Ochratoxin A	<0.2	<0.2	<100

Table 5. Intake and total-tract apparent digestibility of dairy cows fed diets with the inclusion of dietary sugar beet pulp and rice straw mixture silage (BRMS)

Item	Control	Treatment ¹			SEM	P-value ²		
		15BRMS	30BRMS	45BRMS		L	Q	C
Intake (kg/d)								
CP	4.03	4.23	4.40	4.38	0.05	0.02	0.13	0.62
NDF	7.51	7.94	8.30	8.32	0.10	<0.01	0.14	0.67
peNDF ³	5.47	5.51	5.49	5.23	0.05	0.07	0.11	0.64
ADF	4.60	4.80	4.96	4.91	0.05	0.01	0.13	0.62
Apparent digestibility (%)								
DM	63.4	65.2	67.5	65.0	0.52	0.07	0.02	0.16
CP	63.5	66.0	69.5	68.0	0.45	0.07	0.42	0.57
NDF	50.2	52.8	54.6	54.5	0.60	<0.01	0.31	0.78
ADF	42.5	44.6	46.6	46.6	0.55	0.02	0.36	0.60

¹Treatment: 15BRMS, 30BRMS, and 45BRMS indicate the 15%, 30%, and 45% replacement of CS with BRMS, respectively.

²Probabilities of linear (L), quadratic (Q), and cubic (C) effects.

³peNDF = NDF × pef (physically effective fiber) > 1.18 (3 sieve) (Penn State Particle Separator).

Milk Yield and Milk Components

The DMI values and lactation performance of the dairy cows fed the diets with BRMS are presented in Table 8. The DMI values linearly increased with the replacement of CS with BRMS by up to 45% ($P = 0.03$). The enhancing inclusion of dietary BRMS linearly increased the milk yield ($P = 0.048$), and the values of ECM linearly increased with the replacement with BRMS by up to 45% ($P < 0.01$). The milk fat percentage and production were not affected with the increasing inclusion of dietary BRMS ($P = 0.14$ and $P = 0.91$, respectively), and the milk lactose ratio and milk lactose production were not affected ($P = 0.58$ and $P = 0.12$, respectively). The milk protein percentage linearly and quadratically increased with the replacement with BRMS by up to 45% ($P = 0.05$ and $P = 0.04$, respectively), and the milk protein production linearly increased ($P = 0.02$). The MUN linearly decreased as the replacement of BRMS increased up to 45% ($P < 0.01$). The feed efficiencies (milk yield/DMI and FCM/

DMI) were not affected by the diets with an increased inclusion of dietary BRMS ($P = 0.47$ and $P = 0.61$, respectively). In general, the milk yield, milk protein percentage, and production increased as the dairy cows were fed the diets with increased replacement levels of BRMS for CS and this had no unfavorable effects on the health of the animals.

DISCUSSION

The CP content (14.8% DM) of SBP was higher than that in previous reports (Naderi et al., 2016; Kahyani et al., 2019a), the NDF content (51.1% DM) was greater than that in other reports (Kahyani et al., 2019a; Heydari et al., 2021), and the NDF content (75% DM) of rice straw was greater than the result reported by Mu et al. (2020). The differences in nutritional composition may derive from different fertilization harvest seasons, sowing densities, or irrigation methods.

In the current study, the BRM had an appropriate DM content (>250 g/kg of fresh weight; McDonald

Table 6. Effect of replacing corn silage with sugar beet pulp and rice straw mixture silage (BRMS) on rumen fermentation of dairy cows

Item	Control	Treatment ¹			SEM	P-value ²		
		15BRMS	30BRMS	45BRMS		L	Q	C
Total VFA (mM)	96.2	100	103	101	0.93	0.04	0.04	0.46
Acetate (mM)	60.6	62.9	64.3	62.8	0.76	0.26	0.24	0.78
Propionate (mM)	21.6	23.6	24.9	23.9	0.39	0.02	0.05	0.59
Butyrate (mM)	7.23	6.98	7.38	6.88	0.15	0.66	0.70	0.30
Isovalerate (mM)	1.37	1.38	1.37	1.41	0.01	0.22	0.44	0.42
Valerate (mM)	0.42	0.40	0.38	0.39	0.01	0.19	0.41	0.71
Caproic (mM)	2.45	2.48	2.52	2.50	0.01	0.08	0.27	0.48
Acetate:propionate	2.81	2.69	2.59	2.63	0.06	0.25	0.49	0.83
Rumen NH ₃ -N (mg/dL)	12.2	11.9	11.5	11.7	0.09	0.01	0.07	0.05

¹Treatment: 15BRMS, 30BRMS, and 45BRMS indicate the 15%, 30%, and 45% replacement of corn silage with BRMS, respectively.

²Probabilities of linear (L), quadratic (Q), and cubic (C) effects.

Table 7. Effect of replacing corn silage with sugar beet pulp and rice straw mixture silage (BRMS) on the plasma metabolites of dairy cows

Item	Control	Treatment ¹				P-value ²		
		15BRMS	30BRMS	45BRMS	SEM	L	Q	C
Glucose (mg/dL)	4.85	4.90	4.93	4.89	0.02	0.42	0.28	0.78
Triglycerides (mg/dL)	0.51	0.52	0.53	0.52	0.01	0.61	0.57	0.80
BHB (mg/dL)	0.75	0.65	0.50	0.48	0.05	0.02	0.80	0.82
Plasma urea N (mg/dL)	5.65	5.34	5.05	5.15	0.07	0.02	0.18	0.58
Total protein (g/dL)	74.3	75.6	76.8	76.3	0.34	0.01	0.06	0.40

¹Treatment: 15BRMS, 30BRMS, and 45BRMS indicate the 15%, 30%, and 45% replacement of corn silage with BRMS, respectively.

²Probabilities of linear (L), quadratic (Q), and cubic (C) effects.

et al., 1991) and an adequate WSC content (>30 g/kg DM; Zhang et al., 2010a; Guo et al., 2020), which indicated that BRMS meets the silage requirements and has the potential of being used for high quality silage. The WSC content of BRM decreased after silage fermentation, which was mainly due to the substrates (sugar and soluble fiber) utilized by microorganisms to promote the production of lactic acid, since they can rapidly metabolize WSC to lactic acid, leading to an abrupt pH drop. In this study, the pH value of BRMS (3.9) was lower than that of CS, and the value of NH₃-N/total N (3.15) was lower than that of CS, and Catchpoole and Henzell (1971) reported that the fermentation quality of the silages was good, reflecting a lower terminal pH and NH₃-N content, which indicated that the silage quality of BRMS was better than that of CS. The pH value of BRMS was lower than the result reported by Zhang and Zhang (2011), which may be due to the acceleration of the production of lactic acid due

to inoculation. Ogunade et al. (2017) found that a high content of NH₃-N in silage was a marker of excessive protein breakdown, and the value of NH₃-N/total N of BRMS (3.15) in this study was lower than the result reported by Zhang and Zhang (2011), which indicated that the protein breakdown of silage was reduced. The reason might be that the rapid drop of pH induced by inoculation inhibited the proliferation of harmful microorganisms with hydrolysis and deamination (Muck, 1988; Scherer et al., 2015), effectively preventing the production of NH₃-N. In line with our results, Oliveira et al. (2017) analyzed 130 articles and revealed that the silages treated with one or more bacteria showed a lower pH and NH₃-N content compared with those in untreated silages. Few studies have been conducted on BRMS, which limits the comparison of our results with those of other studies.

In this study, the total VFA concentration showed a linear increase with the increasing inclusion of dietary

Table 8. Effects of replacing corn silage with sugar beet pulp and rice straw mixture silage (BRMS) on the DMI, milk yield, milk components, and feed efficiency in dairy cows

Item	Control	Treatment ¹				P-value ²		
		15BRMS	30BRMS	45BRMS	SEM	L	Q	C
DMI (kg/d)	23.7	24.6	25.3	24.9	0.20	0.03	0.13	0.62
Yield milk (kg/d)	39.0	39.8	40.9	40.3	0.29	0.05	0.22	0.40
3.5% FCM ³ (kg/d)	40.1	40.5	41.5	40.6	0.22	0.24	0.13	0.19
ECM ⁴	36.4	37.4	38.8	37.5	0.30	<0.01	0.31	0.03
Fat (kg/d)	1.44	1.44	1.47	1.42	0.01	0.91	0.27	0.26
Protein (kg/d)	1.15	1.26	1.35	1.27	0.02	0.02	0.02	0.32
Lactose (kg/d)	1.97	2.02	2.08	2.05	0.01	0.12	0.31	0.61
Composition								
Fat (%)	3.68	3.62	3.60	3.54	0.03	0.14	1.00	0.78
Protein (%)	2.95	3.16	3.29	3.15	0.05	0.05	0.04	0.58
Lactose (%)	5.04	5.08	5.09	5.08	0.02	0.58	0.68	0.96
MUN (mg/dL)	11.3	11.0	10.5	10.5	0.11	<0.01	0.01	0.60
Feed efficiency								
Milk yield/DMI	1.69	1.64	1.66	1.65	0.01	0.47	0.51	0.40
FCM/DMI	1.53	1.52	1.55	1.53	0.01	0.61	0.74	0.24

¹Treatment: 15BRMS, 30BRMS, and 45BRMS indicate the 15%, 30%, and 45% replacement of corn silage with BRMS, respectively.

²Probabilities of linear (L), quadratic (Q), and cubic (C) effects.

³FCM = 0.432 × milk yield + 16.23 × fat yield.

⁴ECM = milk production × (0.383 × %fat + 0.242 × %protein + 0.7832)/3.1183 (NRC, 2001).

BRMS. Moreover, Weiss et al. (2009) demonstrated that a high concentration of total VFA was an energy utilization efficiency indicator, which suggested that the inclusion of dietary BRMS increased the energy utilization efficiency. Similar to our results, Heydari et al. (2021) reported that the total VFA in the rumen increased with the increasing inclusion of dietary BP and proposed that the increase in fermentable substrates promoted the proliferation of ruminal microbes. The dietary insufficient energy induced by a low starch content might be compensated for by an increased soluble fiber, sugar, and fiber digestibility, and Silva et al. (1989) demonstrated that the decrease in the total VFA concentration induced by inhibiting the proliferation of rumen microbes was due to the insufficiency of soluble or digestible fiber. Additionally, inoculation was probably another reason for the increase in the total VFA, and Guo et al. (2020) reported that the silage inoculated with LAB had a higher potential of gas production than that did untreated silage.

In this study, the propionate concentration in the rumen had a linear increase with the increasing inclusion of dietary BRMS, which was consistent with the well-documented effect of a decreasing peNDF content and an increasing NDF digestibility (Maktabi et al., 2016; Naderi et al., 2016). The propionate was the main glucogenic precursor in providing energy for milk synthesis, which indicated that the milk yield might increase with the inclusion of dietary BRMS. In this study, the acetate concentration in the rumen was kept constant with the increasing inclusion of dietary BRMS because acetate concentration was positively correlated with the synthesis of milk fat (Heydari et al., 2021), which is consistent with an unchanged milk fat percentage and production. However, Naderi et al. (2016) found that the acetate concentration decreased with the inclusion of dietary BP, and the differences may be due to BP processing via shredding, which decreased the chewing activity by reducing the dietary peNDF content. In this study, the inclusion of dietary rice straw in the mixture silage compensated for the insufficiency of peNDF, which was consistent with the unchanged peNDF intake. No significant differences were observed in the acetate-to-propionate ratios, which was consistent with the results obtained by Hao et al. (2017).

The ruminal $\text{NH}_3\text{-N}$ concentration linearly decreased with the increasing replacement of BRMS in the diets, which might be explained by an increased NDF digestibility to increase the utilization of ammonia by rumen microbes, and MUN data would support that. Inoculation might be another reason for the decrease in the ruminal $\text{NH}_3\text{-N}$ concentration, and Guo et al. (2020) reported that corn stover silage inoculated with LAB had a lower ruminal $\text{NH}_3\text{-N}$ concentration than

that did untreated silage. The reason for the decreased ruminal $\text{NH}_3\text{-N}$ concentration was that the fermentation efficiency (the speed of acidification) was improved by adding LAB during the initial silage fermentation phase (Muck, 1988; McDonald et al., 1991), which reduced the activity of microbial protease. In this study, the plasma concentration of BHB decreased with the inclusion of dietary BRMS, which was consistent with the results by Naderi et al. (2016). The plasma concentration of UN decreased as the inclusion of dietary BRMS increased up to 45%, which indicated that the energy and protein levels were more balanced with an increasing level of BRMS, since a low UN concentration reflected a high nitrogen efficiency. Wang et al. (2019) demonstrated the increase of TP strengthened tissue protein synthesis and organ growth, and the TP linearly increased as the BRMS increased up to 45%, which indicated that the dietary inclusion of BRMS enhanced nitrogen efficiency of dairy cows.

In this study, the DMI values of high-producing dairy cows increased with the increasing inclusion of dietary BRMS; similarly, Kargar et al. (2019) reported that the substitution of BP for CS and alfalfa hay had a positive effect on the feed intake, and Heydari et al. (2021) found that a high BP content in the diet caused a linear increase in DMI and NDF digestibility. Kahyani et al. (2019b) found that the WSCS diet (inclusion of BP) contributed to a greater DMI because BP had a high degradable NDF content and a high NDF digestibility, as well as a high cation exchange capacity, and Fustini et al. (2016) demonstrated that a diet with a high degradable NDF content and a high NDF digestibility was the main reasons for an increased DMI. Additionally, the good palatability of BRMS with inoculation was also an important reason (metabolites such as organic acids), and Beiranvand et al. (2019) demonstrated that a high dietary lactic acid content increased the palatability.

The total-tract digestibility of DM, NDF, and ADF increased with the replacement with BRMS by up to 45%. In line with our results, Kahyani et al. (2019b) reported that the DM and NDF digestibility values were improved by the inclusion of dietary BP in high-producing cows, and Heydari et al. (2021) demonstrated that the DM and NDF digestibility values of Holstein dairy cows in mid lactation tended to linearly increase with a higher BP. The NDF digestibility was an important target for improving energy utilization in dairy cows (Reynolds et al., 2011), and an increased NDF digestibility promoted the proliferation of microorganisms and improved the capacity of production of digestive enzymes by microorganisms, thereby further decomposing the nutrients to improve their utilization. Zhang et al. (2010b) found that nutrient digestibility

was improved by the proliferation of white rumen cocci, yellow rumen cocci, and succinate filamentous fungi.

In this study, the improvement in milk yield was related to the increasing inclusion of dietary BRMS; similarly, Naderi et al. (2016) found that the inclusion of dietary BP improved the milk yield. The improvement in milk yield might be due to high DMI and DM digestibility, which represent a high utilization efficiency of energy and protein. Another possible explanation for the increased milk yield is the change of the ruminal fermentation profile (the increase in propionate concentration). Propionate is the main glucogenic precursor for milk synthesis (Ramanzin et al., 1997). No significant reduction was observed in the milk fat percentage, which was consistent with the constant concentrations of acetate and butyrate. According to the NRC (2001), the ration of a lactating cow should contain at least 200 g/kg peNDF, and the peNDF for the replacement diets in this study were more than 200 g/kg DM. Therefore, the effective fiber was adequate to keep constant milk fat percentage.

The milk protein production increased with the increasing replacement levels of BRMS in the diets, which was consistent with the results obtained by Naderi et al. (2016), where it was reported that the milk protein production of dairy cows fed an increasing dietary concentration of SBP at the expense of CS linearly increased ($P = 0.03$). The increase in milk protein production may be due to an increased NDICP content with the BRMS replacement increasing in the diets. The increased NDICP content promoted the flow of MP to the small intestine, thereby enhancing the efficiency of nitrogen utilization, which led to a decrease in the ruminal $\text{NH}_3\text{-N}$ concentration and to a corresponding decrease in MUN (Naderi et al., 2016). In line with our results, Naderi et al. (2016) reported that the MUN concentration decreased as the replacement levels of dietary BRMS increased, since a low MUN concentration indicated the high efficiency of synthetic bacterial protein (Farmer et al., 2014), which suggested that the protein utilization efficiency increased due to the balance of ruminal energy and protein. In addition, the increase in the dietary CP intake was also an important reason for the improvement in milk protein production.

The values of feed intake, milk yield, and milk protein production of high-production dairy cows were improved by the partial replacement of CS with BRMS in the diets, and the optimal inclusion ratio of BRMS in place of CS in the diets of high-producing dairy cows was determined to be 45%. The 45% inclusion in the diet was likely to be a result of a better balance of energy and protein.

CONCLUSIONS

Feeding diets with the inclusion of BRMS in place of a portion of CS linearly improved the total-tract digestibility and lactation performance of high-production lactating dairy cows, and the effects of replacing levels of up to 45% were optimal and had no unfavorable effects on the health of the animals. The decreased cost and reduced air pollution associated with BRMS are benefits of its widespread application.

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