Effect of substituting brown rice for corn on lactation and digestion in dairy cows fed diets with a high proportion of grain

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ABSTRACT

The effects of the substitution of brown rice (*Oryza sativa* L.; BR) for corn (*Zea mays* L.) in ensiled total mixed ration (TMR) that had a high proportion of grain on feed intake, lactation performance, ruminal fermentation, digestion, and N utilization were evaluated. Nine multiparous Holstein cows (51 ± 9 d in milk) were used in a replicated 3×3 Latin square design with 3 dietary treatments: a diet containing 0, 20, or 40% steam-flaked BR and 40, 20, or 0% steam-flaked corn (dry matter basis). Cows were fed ad libitum an ensiled TMR consisting of 40.7% alfalfa silage, 11.8% grass silage, 7.1% soybean meal, and 40.0% steam-flaked grain (dry matter basis). The ensiled TMR was prepared by baling fresh TMR, and then sealed by a bale wrapper and stored outdoors at 5 to 30°C for over 6 mo. Dry matter intake and milk yield were lower for cows fed 40% BR than for cows fed 40% corn. The ruminal pH and total volatile fatty acid concentrations were not affected by dietary treatment. The ruminal ammonia-N concentration decreased as the percentage of BR in the diets was elevated. The proportion of acetate decreased, and that of propionate and butyrate increased with the increasing levels of BR. Plasma urea-N concentrations were lower and glucose and insulin concentrations were higher for cows fed 40% BR than for cows fed 40% corn. The whole-tract apparent digestibility of dry matter, organic matter, and starch increased, and the digestibility of neutral detergent fiber and acid detergent fiber decreased with the increasing BR level in the diet, with no dietary effect on crude protein digestion. As a proportion of N intake, the urinary N excretion was lower and the retention of N was higher for cows fed 40% BR than for cows fed 40% corn, with no dietary effect observed on N secretion in milk and fecal N excretion. These results show that substituting BR for corn decreases urinary N losses and improves N utilization, but causes adverse effects on milk production

when cows are fed high-grain diets at 40% of dietary dry matter.

Key words: brown rice, dairy cow, ensiled total mixed ration, nitrogen balance

INTRODUCTION

Corn grain is used as the main dietary starch source in dairy farming. In Japan, however, brown rice grain (BR) has recently begun to be used in dairy cow diets as a partial substitute for corn grain due to high prices of imported corn grain, and the cultivation area of rice grain for feed has been increasing (MAFF, 2013a). Japan's National Agriculture and Food Research Organization (NARO, 2009) reported that the digestible and metabolizable energy of BR are 4.2 and 3.7 Mcal/kg and that those of corn grain are 4.1 and 3.6 Mcal/kg, respectively. Miyaji et al. (2012) reported the concentrations of CP, NDF, ADF, and starch as 7.5, 7.7, 2.8, and 67.4% for steam-flaked corn and 7.5, 5.3, 1.6, and 70.9% for steam-flaked BR (DM basis), respectively. Based on these similar energy and nutrient contents between corn grain and BR, BR is a potentially suitable replacement for corn grain in dairy cow diets.

The characteristics of ruminal starch degradation vary with the cereal grain sources (Huntington, 1997), and BR starch is digested in the rumen more rapidly than corn grain starch (Miyaji et al., 2012). In dairy cows, these differences in ruminal digestion characteristics due to the dietary starch source have a large impact on DMI (McCarthy et al., 1989), digestion (Harmison et al., 1997), milk fat concentration (Grummer, 1988), and N utilization (Krause et al., 2002; Cabrita et al., 2006; Gozho and Mutsvangwa, 2008). Miyaji et al. (2012, 2013) reported that the replacement of corn with BR had little effect on DMI, milk yield, and composition when cows were fed diets that contained 30.9 to 31.2% grain (DM basis). On the other hand, Oba and Allen (2003) reported that replacing the starch source that has a lower ruminal digestibility with one that has a greater ruminal digestibility resulted in DMI depressions and decreased the productivity of cows fed diets containing a high percentage of grain. Thus, increasing the ruminal starch digestibility by substituting BR for

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corn could cause adverse effects on the production of lactating cows fed diets containing a high proportion of grain. However, little research has been conducted to evaluate the effects of the substitution of BR for corn on the digestion and lactation performance of dairy cows when cows are fed diets with a high proportion of grain.

We hypothesized that differences in the ruminally digested starch content between corn and BR would result in differences in the productivity, ruminal fermentation, and N utilization when cows were fed diets with a high proportion of grain. Therefore, the objective of the present study was to evaluate the effect of substituting steam-flaked BR for steam-flaked corn on DMI, milk production, ruminal fermentation, blood metabolites, nutrient digestibility, and N balance in cows fed diets containing grain at 40% of dietary DM. Additionally, the practice of using ensiled TMR as dairy cow diets is increasing in Japan by increasing the number of TMR centers (MAFF, 2013b). The ensiled TMR has some advantages, such as the supply of homogeneous feed over time to cows, the labor savings during preparation of TMR, the opportunity to include wet food by-product, and the improvement in aerobic stability (Nishino et al., 2003; Wang and Nishino, 2008; Xu et al., 2007, 2010). Thus, in the present study, the experimental diet was fed as an ensiled TMR.

MATERIALS AND METHODS

All animal studies were conducted in accordance with the animal care and use guidelines of the National Agriculture and Food Research Organization (NARO) Institute of Livestock and Grassland Science of Japan (Nasushiobara, Japan).

Cows, Diets, and Management

Nine multiparous Holstein cows (3.0 ± 1.1 parity, 578 ± 68 kg of BW, 51 ± 9 DIM) were used in a replicated 3×3 Latin square design with 3 dietary treatments. The experimental period was 21 d, with 14 d for treatment adaptation and 7 d for data collection. The diets were formulated with 52.5% forage in combinations of alfalfa silage and grass silage, and 47.5% concentrate in combinations of steam-flaked cereal grain, soybean meal, minerals, and vitamins (DM basis). The dietary treatments were a diet containing 40.0% steam-flaked corn, 40.0% steam-flaked BR, or an equal mixture of steam-flaked corn and BR (Table 1). Both cereal grains were steamed under similar conditions (100°C for 20–30 min) and flaked. The densities of grains were measured in triplicate by weighing a standard volume (2 L) of processed grains. The volume weights of the processed

corn and BR were 52.7 and 59.3 kg/100 L, respectively. The processing index of corn and BR, which was expressed volume weight after processing as a proportion of volume weight before processing, was 73.7 and 77.2, respectively. All experimental diets were formulated to meet or exceed the Japanese Feeding Standard for Dairy Cattle (NARO, 2006). The diets were fed as ensiled TMR. The ensiled TMR was prepared by baling fresh TMR using a round baler for chopped material (TSB1000; IHI STAR Machinery Corp., Chitose, Japan). The TMR was then sealed by a bale wrapper and stored outdoors at 5 to 30°C after preparation for over 6 mo. Throughout the experiment, the cows were housed in individual tie-stalls and had free access to fresh water. The cows were offered the diets for ad libitum intake (10% refusals) twice daily (0900 and 1900 h) and were milked in their stalls twice daily (0830 and 1830 h).

Data and Sample Collection

During d 15 to 20 of each period, the amount of feed offered andorts were weighed and recorded daily. Representative samples of ensiled TMR and orts were collected daily during d 15 to 20 of each period. The ensiled TMR sample was stored at -30°C for later

Table 1. Ingredient proportions, chemical composition, and fermentation characteristics of experimental ensiled TMR

Item	Corn:rice		
	40:0	20:20	0:40
Ingredient, % of DM			
Alfalfa silage	40.7	40.7	40.7
Grass silage	11.8	11.8	11.8
Steam-flaked brown rice	0.0	20.0	40.0
Steam-flaked corn	40.0	20.0	0.0
Soybean meal	7.1	7.1	7.1
Dicalcium phosphate	0.1	0.1	0.1
Mineral mix ¹	0.2	0.2	0.2
Vitamin mix ²	0.2	0.2	0.2
Chemical composition			
DM, %	49.2	49.6	49.4
OM, % of DM	92.8	92.9	92.8
CP, % of DM	18.5	18.5	18.6
Ether extract, % of DM	4.2	3.6	3.2
NDF, % of DM	33.6	31.8	31.1
ADF, % of DM	25.4	25.3	25.2
Starch, % of DM	25.4	26.5	27.6
Fermentation profile			
pH	3.90	3.78	3.72
Lactic acid, % of DM	8.70	9.36	9.63
Acetic acid, % of DM	2.08	1.91	1.96
Propionic acid, % of DM	0.11	0.09	0.10
Butyric acid, % of DM	0.06	0.07	0.06
Volatile basic N, % of DM	0.13	0.12	0.12

¹Contained 220 g of Ca/kg, 110 g of P/kg, and 100 g of Mg/kg.

²Contained 5,000,000 IU of vitamin A/kg, 600,000 IU of vitamin D₃/kg, and 20,000 mg of DL- α -tocopherol acetate/kg.

determination of the chemical composition and the fermentation products. The ort samples were pooled, and 1 sample was retained per cow per period. The ensiled TMR samples were freeze-dried and the ort samples were dried at 60°C in an oven for 48 h. The dried samples were ground through a 1-mm screen using a centrifugal mill (ZM200; Retsch GmbH, Haan, Germany) and stored until analysis. The milk yield was measured daily and averaged over the collection period, and duplicate milk samples were taken at every milking. One set of duplicate samples was analyzed for fat, protein, lactose, and urea-N concentrations by infrared spectroscopy (MilkoScan 133B; Foss Electric A/S, Hillerød, Denmark). The second set of samples was refrigerated until the end of each collection period, composited by cow within the period according to milk production and frozen at -30°C until N analysis.

On d 15 to 20 of each period, whole-tract apparent digestibility and N balance were determined by the total collection of feces and urine using an automatic feces-urine separating machine (Nishida et al., 2007). All feces were collected daily in buckets. The total weight of the daily feces was measured, and a portion of 5% of total weight was then stored at -20°C during the collection period. Just after collection, feces samples were mixed well. A portion of fresh feces was dried at 60°C for at least 72 h in a forced-air oven and then ground through a 1-mm screen, and the other portion was used for analyzing the DM and total N. All urine was collected daily in plastic buckets containing 250 mL of 20% sulfuric acid that had been added to achieve urine pH of less than 3.0. The total weight of the urine was measured, and a sample of 5% of the total weight was stored in a refrigerator at 5°C during the collection. Just after collection, urine samples were mixed well and stored at -30°C until analysis.

Ruminal fluid was collected via an esophageal tube using a rumen catheter (Sanshin Industrial Co. Ltd., Yokohama, Japan) at 0 (just before the morning feeding), 2, 4, 6, 8, and 10 h after the morning feeding on d 21 of each period. Ruminal fluid pH was measured by a glass electrode pH meter (D-51; Horiba Ltd., Kyoto, Japan) immediately after sample collection. The ruminal fluids were separated from the feed particles through 4 layers of gauze and centrifuged at $1,200 \times g$ for 15 min at 5°C. For the determination of VFA and ammonia-N concentrations, 1 mL of 30% perchloric acid solution as a deproteinizing agent was added to 4 mL of ruminal fluid, and the resultant fluid samples were then stored at -30°C until analysis. Blood samples were collected into heparinized tubes from the jugular vein at 0 and 4 h after the morning feeding on d 21 of each period. The samples were immediately centrifuged at $1,200 \times g$

for 15 min at 5°C, and the plasma was stored at -30°C until the urea-N, glucose, and insulin analysis.

Chemical Analysis

The DM of the feed and orts was determined by drying the sample at 105°C for 24 h; the wet fecal samples were dried at 135°C for 2 h after drying at 60°C for 24 h. Wet feces were used for analyzing nitrogen. The OM was calculated as the difference between the DM and ash contents after the ash determination by the AOAC International method (AOAC International, 2000; method 942.05). The ether extract and Kjeldahl N contents were determined by AOAC International methods (AOAC International, 2000; methods 920.39 and 990.03, respectively). The NDF and ADF (NDF was assayed with a heat-stable amylase and sodium sulfite and expressed exclusive of residual ash; ADF was expressed exclusive of residual ash) were analyzed by the methods of Van Soest et al. (1991). The starch was analyzed using a starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland; McCleary et al., 1994). The urine and milk samples were analyzed for Kjeldahl N with the same methodology as that used for the feeds. The urine samples were analyzed for allantoin by the colorimetric method described by Chen and Gomes (1992) and for uric acid using a commercial kit (Uric acid C-test; Wako Pure Chemical Industries, Osaka, Japan). The microbial N supply to the small intestine was calculated from the urinary output of purine derivatives using the equation of Chen and Gomes (1992).

Fermentation products of the ensiled TMR were determined from cold-water extracts. Wet ensiled TMR (60 g) was homogenized with 140 mL of sterilized distilled water and stored at 4°C overnight (Cai et al., 1999). The pH was measured with a glass electrode pH meter (D-51; Horiba Ltd.), and volatile basic nitrogen concentration was determined by steam distillation of the filtrates. The organic acid contents were measured by HPLC (Hosoda et al., 2006).

The samples of deproteinized ruminal fluid were neutralized with potassium hydroxide solution and centrifuged at $1,200 \times g$ for 10 min. The supernatants were shaken with a cation-exchange resin (Amberlite, IR 120B H AG; Organo Corp., Tokyo, Japan) and centrifuged at $6,500 \times g$ for 5 min at 5°C. The supernatants were used for the analysis of lactic acid and VFA using HPLC as they were for the ensiled TMR filtrates, and for the analysis of ammonia-N (Weatherburn, 1967). Urea-N and glucose in plasma were measured with an automatic analyzer (7080 Clinical Analyzer; Hitachi Co., Tokyo, Japan). A commercial kit (bovine insulin

Table 2. Effect of substituting brown rice for corn on DMI, milk yield, and milk composition in dairy cows

Item	Corn:rice			SEM	P-value
	40:0	20:20	0:40		
DMI, kg/d	24.8 ^a	24.0 ^{ab}	23.5 ^b	0.12	0.01
Yield, kg/d					
Milk	44.8 ^a	43.7 ^a	42.0 ^b	0.32	0.001
Milk fat	1.61	1.52	1.54	0.002	0.21
Milk protein	1.35 ^a	1.32 ^{ab}	1.27 ^b	0.001	0.03
Milk lactose	2.09 ^a	2.03 ^a	1.94 ^b	0.001	<0.001
Milk composition					
Fat, %	3.61	3.51	3.70	0.008	0.20
Protein, %	3.01	3.03	3.02	0.001	0.86
Lactose, %	4.67	4.64	4.61	<0.001	0.14
Urea-N, mg/dL	17.4 ^a	16.6 ^{ab}	15.9 ^b	0.23	0.03

^{a,b}Means within a row without a common superscript letter differ ($P < 0.05$).

ELISA kit; Mercodia AB, Uppsala, Sweden) was used to determine the plasma insulin concentration.

Statistical Analysis

Data on all variables were analyzed using PROC MIXED of SAS (version 9.2; SAS Institute, 2004). Data on DMI, milk yield, milk composition, digestibility, and N balance were analyzed as replicated 3×3 Latin squares using the following model: $Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + \varepsilon_{ijkl}$, where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i , P_j is the fixed effect of period j , $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of treatment l , and ε_{ijkl} is the random residual error. Measurements of the ruminal pH; lactic acid, VFA, and ammonia-N concentrations; and the plasma concentration were analyzed as repeated measures. The statistical model used included square, period, treatment, time, and treatment $\times$ time interaction as fixed effects, and cow as a random effect. The multiple comparisons among the dietary treatments were carried out using the Tukey method, and significance was declared at $P < 0.05$.

RESULTS

Diet Characteristics

Across the diets, the DM, OM, and CP contents were 49.4, 92.8, and 18.5% (DM basis), respectively (Table 1). The starch content of the ensiled TMR increased and the ether extract and NDF contents decreased with increasing levels of BR, which is a reflection of the differences in the nutrient contents of the original grains. All ensiled TMR were well preserved, with low pH value, volatile basic nitrogen, and butyric acid contents and high lactic acid content. The pH value decreased

and the lactic acid concentration increased as the inclusions of BR in the ensiled TMR increased.

DMI and Milk Production

Dry matter intake ($P = 0.01$) and milk yield ($P < 0.001$) were affected by dietary treatments: both decreased with an increasing BR level in the diet (Table 2). Milk protein ($P = 0.03$) and lactose ($P < 0.001$) yields were also affected by dietary treatments and decreased in response to increased levels of BR. The milk fat yield and the milk contents of fat, protein, and lactose were not affected by the dietary treatments. A significant difference among the dietary treatments in milk urea-N content was observed ($P = 0.03$), and was lower for cows fed 40% BR than for cows fed 40% corn.

Ruminal Fermentation Characteristics and Plasma Metabolites

No significant interactions were observed between dietary treatments and time for all ruminal fermentation characteristics and plasma metabolites (Table 3). Ruminal pH was not affected by dietary treatment, averaging 6.58 across the 3 diets. Neither rumen lactic acid nor the total VFA concentrations was influenced by the replacement of corn with BR. The molar proportion of acetate ($P < 0.002$), propionate ($P = 0.04$), isobutyrate ($P < 0.002$), butyrate ($P = 0.04$), and valerate ($P < 0.001$) were affected by dietary treatment: those of acetate and isobutyrate were lower and those of propionate, butyrate, and valerate were higher for cows fed 40% BR than for cows fed 40% corn. Significant differences were observed among dietary treatments in the concentrations of rumen ammonia-N ($P = 0.04$) and plasma urea-N ($P = 0.04$): both were decreased with the increasing BR level. Significant differences among the dietary treatments in plasma glucose ($P = 0.01$)

Table 3. Effect of substituting brown rice for corn on ruminal fermentation characteristics and plasma metabolites in dairy cows

Item	Corn:rice			SEM	P-value		
	40:0	20:20	0:40		Diet	Time	Diet × time
Ruminal fermentation characteristics							
pH	6.58	6.60	6.55	0.008	0.78	<0.001	0.96
Ammonia-N, mg/dL	11.23 ^a	10.35 ^{ab}	8.91 ^b	3.100	0.04	<0.001	0.64
Lactic acid, mM	3.9	3.7	6.6	1.00	0.31	0.001	0.94
Total VFA, mM	112.7	112.4	113.4	16.47	0.68	0.02	0.68
VFA, mol/100 mol							
Acetate	63.5 ^a	62.3 ^{ab}	61.3 ^b	0.51	0.002	0.12	0.71
Propionate	22.1 ^b	23.2 ^{ab}	23.4 ^a	0.35	0.04	0.08	0.42
Isobutyrate	0.9 ^a	0.8 ^{ab}	0.7 ^b	<0.01	0.002	0.02	0.68
Butyrate	10.1 ^b	10.5 ^{ab}	10.9 ^a	0.09	0.04	0.63	0.92
Isovalerate	1.6	1.4	1.5	0.01	0.09	0.25	0.48
Valerate	1.8 ^b	1.9 ^b	2.2 ^a	0.01	<0.001	0.123	0.98
Plasma metabolite							
Urea-N, mg/dL	13.2 ^a	12.3 ^{ab}	11.8 ^b	0.29	0.04	0.43	0.31
Glucose, mg/dL	63.3 ^b	63.8 ^b	67.4 ^a	3.31	0.01	0.06	0.86
Insulin, mIU/L	8.7 ^b	12.3 ^{ab}	14.4 ^a	0.36	0.04	0.23	0.80

^{a,b}Means within a row without a common superscript letter differ ($P < 0.05$).

and insulin ($P = 0.04$) concentrations were observed, and both were higher for cows fed 40% BR than for cows fed 40% corn.

Whole-Tract Digestibility and Nitrogen Balance

No significant differences existed in apparent CP and ether extract digestibility among the dietary treatments (Table 4). The whole-tract apparent digestibility of DM ($P < 0.001$), OM ($P < 0.001$), NDF ($P < 0.001$), ADF ($P < 0.005$), and starch ($P < 0.001$) were affected by dietary treatments. The DM, OM, and starch digestibilities were increased with the increasing BR level in the diet. Conversely, the NDF and ADF digestibilities were decreased as the BR in the diets increased.

The nitrogen intake was affected by dietary treatments ($P = 0.03$), and decreased with the increased levels of BR, which is a reflection of decreasing DMI (Table 5). The significant differences among the dietary treatments in secreted N in milk ($P = 0.04$), urinary N excretion ($P < 0.001$), and retained N ($P = 0.02$) were observed with no dietary effect on fecal N excretion.

The milk N secretion and urinary N excretion were lower, and the N retention was higher for cows fed 40% BR than for cows fed 40% corn. However, as a proportion of N intake, the milk N secretion and fecal N excretion were not affected by the dietary treatments, and the significant differences in urinary N excretion ($P < 0.001$) and N retention ($P < 0.007$) were detected. As a proportion of N intake, urinary N excretion decreased and N retention increased with the increasing BR. The urinary allantoin, uric acid, and total purine derivative excretions, and calculated intestinal flow of microbial N were not affected by the substitution of BR for corn.

DISCUSSION

It has been shown that the DMI decreased when cows were fed more rapidly available starch sources (McCarthy et al., 1989; Krause et al., 2002), and our present results are consistent with those reports, because here the BR starch was digested more rapidly than the corn starch in the rumen. Oba and Allen (2003) reported that these DMI depressions obtained by replacing the

Table 4. Effect of substituting brown rice for corn on whole-tract digestibility (%) in dairy cows

Item	Corn:rice			SEM	P-value
	40:0	20:20	0:40		
DM	69.3 ^c	70.3 ^b	71.4 ^a	0.11	<0.001
OM	70.0 ^c	71.2 ^b	72.4 ^a	0.11	<0.001
CP	66.7	66.0	65.9	0.18	0.20
Ether extract	72.1	71.8	73.8	1.66	0.32
NDF	51.1 ^a	49.1 ^b	47.0 ^c	0.33	<0.001
ADF	53.7 ^a	53.0 ^a	51.2 ^b	0.37	0.005
Starch	96.0 ^c	97.4 ^b	99.0 ^a	0.09	<0.001

^{a-c}Means within a row without a common superscript letter differ ($P < 0.05$).

Table 5. Effect of substituting brown rice for corn on nitrogen utilization in dairy cows

Item	Corn:rice			SEM	P-value
	40:0	20:20	0:40		
N balance					
N intake, g/d	736.8 ^a	712.6 ^{ab}	702.3 ^b	113.43	0.03
Milk N					
g/d	211.4 ^a	205.0 ^{ab}	198.2 ^b	22.02	0.04
% of N intake	28.8	28.8	28.3	0.22	0.60
Fecal N excretion					
g/d	245.3	242.4	239.3	37.77	0.67
% of N intake	33.3	34.0	34.1	0.18	0.20
Urinary N excretion					
g/d	204.9 ^a	184.3 ^b	173.9 ^b	17.62	<0.001
% of N intake	27.8 ^a	25.8 ^b	24.7 ^b	0.28	<0.001
N retention					
g/d	75.2 ^b	80.9 ^b	90.9 ^a	19.05	0.02
% of N intake	10.2 ^b	11.4 ^{ab}	12.9 ^a	0.48	0.007
Urinary excretion, mM/d					
Allantoin	421.6	437.2	438.0	29.26	0.24
Uric acid	46.8	64.5	57.3	10.93	0.62
Total PD ¹	468.4	501.7	495.3	38.93	0.28
Microbial N supply, ² g/d	354.5	381.9	376.7	37.89	0.28

^{a,b}Means within a row without a common superscript letter differ ($P < 0.05$).

¹PD = purine derivatives: allantoin and uric acid.

²Calculation based on equation from Chen and Gomes (1992).

starch source with greater ruminal degradation might be explained by a higher propionate production in the rumen. Additionally, Reynolds et al. (2003) and Allen et al. (2009) suggested that the formulation diets to shift starch digestion to the small intestine not only decreased propionate production, but also increased lactate absorption from glucose metabolism in the gut, and thus this shift resulted in greater feed intake. In the present study, the ruminal propionate concentration was increased by substituting BR for corn, although the differences among dietary treatments were quite small. Thus, increasing ruminal starch digestibility and ruminal propionate production by substituting BR for corn might cause DMI depression. On the other hand, Yang et al. (2000), investigating the impact of increasing ruminal starch degradation, had attributed a decrease in DMI to the induction of ruminal acidosis. In the present study, mean ruminal pH was not affected by dietary treatment, but this was based on spot sampling of ruminal fluid collected via an esophageal tube. The extent and severity of ruminal acidosis would be obtained with continuous pH measurements. The greater concentrations of ruminal lactate with 40% BR might be indicative of induction of ruminal acidosis, which ruminal pH measurements based on spot sampling might have failed to detect. Based on these factors, it is likely that the lower DMI with BR could be attributable to a multifactorial response, such as higher molar propionate concentration and ruminal acidosis. Miyaji et al. (2012), after feeding lactating cows 0, 15.6, and 31.2% steam-flaked BR as dietary DM in place of steam-flaked corn

with around 35% dietary starch, reported that the DMI was not different among the 3 treatments. Miyaji et al. (2013) also found that the replacement of corn with BR did not affect the DMI in cows fed diets consisting of 30.9% steam-flaked grain (DM basis) with around 20% dietary starch. These previous findings are inconsistent with the present results. de Visser et al. (1990) and Grings et al. (1992) suggested that the effects of grain source on DMI can be attributed to the proportion of grain in the diets. Additionally, Oba and Allen (2003) reported that replacing the starch source with greater ruminal digestibility resulted in DMI depressions when the cows were fed diets containing a high, but not low, inclusion of grain. The proportion of grain inclusion in the TMR in the present study was greater (40.0 vs. 31.2 and 30.9% of dietary DM) than those in the Miyaji et al. (2012, 2013) studies. Therefore, DMI depression caused by replacing corn with BR would occur in cows fed diets with a high proportion of grain.

Miyaji et al. (2012, 2013) reported that replacing corn with BR did not affect milk yield. In contrast, in the present study, the milk yield decreased in response to the increased level of BR. The DMI depression caused by replacing corn with BR occurred in the present study, but not in the previous studies of Miyaji et al. (2012, 2013). We therefore attribute the reduction in milk yield to the decreased DMI that occurred with the increased BR in the diet. On the other hand, the increased digestibility of DM due to replacing corn with BR could be expected to cancel the milk yield depression. However, the digestible DM, OM, NDF, and

starch intake were decreased by replacing corn with BR; thus, the higher DM digestibility could not offset the milk yield. The concentrations of milk fat, lactose, and protein were not affected by the substitution of BR for corn, a result that is consistent with those of Miyaji et al. (2012, 2013).

The ruminal starch disappearance rate and the effective degradability of BR, determined by the *in situ* technique, were higher than that of corn (Miyaji et al., 2012). Thus, greater ruminal starch digestibility for BR could be expected to linearly decrease ruminal pH and increase the lactic acid concentrations as the proportion of BR is increased in the diet. However, the dietary treatment in the present study did not influence the ruminal pH and lactic acid concentrations, a result that is consistent with those of Miyaji et al. (2012, 2013). In the present study, mean ruminal pH was based on spot sampling of ruminal fluid collected via an esophageal tube. Because of contamination of saliva, ruminal pH measurements based on collection via an esophageal might have failed to detect differences among treatments. The total VFA concentration was not affected by the level of BR, but the rumen fermentation pattern changed when corn was replaced with BR. The decreased acetate and increased propionate and butyrate concentrations with the increasing level of BR could be attributed to the high starch content and rapid starch degradability of BR compared with corn. In the present study, the diet was fed as an ensiled TMR. The ensiling process could be expected to solubilize endosperm protein and increase starch degradation; thus, the ruminal fermentation of cows fed ensiled TMR used in this study could be greater compared with cows fed unensiled fresh TMR. The change in the nutrients and ruminal degradation characteristics in ensiled TMR during storage are not yet clear and, thus, need to be considered.

The plasma glucose concentration arises largely from hepatic gluconeogenesis using ruminally derived propionate as the principal precursor (Huntington, 1997). In fact, van Knegsel et al. (2005, 2007) and Cabrita et al. (2007) showed that feeding dairy cows increased levels of glycogenic nutrients could increase their plasma glucose or insulin concentration, or both. Thus, the increase in the plasma glucose and insulin concentrations as the proportion of BR was increased in the diet could be attributed to the greater starch ruminal fermentation and the higher concentration of ruminal propionate in BR-based ensiled TMR.

The increased digestibilities of DM, OM, and starch due to the substitution of BR for corn could be caused by the greater rate of ruminal DM and starch degradation of BR compared with those of corn. Miyaji et al. (2012, 2013) found similar results for DM, OM, and

starch digestibility when corn was replaced with BR in the diet. However, the effects of diet on fiber digestibility were different from those observed for DM, as the digestibilities of NDF and ADF were decreased linearly when BR in the diet increased. Harmison et al. (1997) reported that the high rate of cereal digestion could increase the likelihood of decreasing fiber digestibility caused by lowered ruminal pH. Although the clear reduction of ruminal pH was not detected in the present study, the rapid starch degradation of BR could damage the ruminal fiber fermentation and then decrease total-tract fiber digestion with increasing BR.

With increasing BR in the diet, the decrease in urinary N excretion and the increase in N retention with no dietary effect on secreted N in milk and on fecal N excretion as a proportion of N intake suggests that the efficient use of dietary CP would be improved by the replacement of corn with BR in diets, and these results are consistent with those of Miyaji et al. (2012, 2013). Increasing the availability of ruminal fermentable starch increased the ruminal microbial protein synthesis (Theurer et al., 1999; Krause et al., 2002). The substitution of BR for corn might improve ruminal N use by providing a more available starch source for microbial protein production, and this improvement might decrease the urinary N losses in dairy cows. The decreased ruminal ammonia-N and plasma urea-N concentrations with increasing BR may support this hypothesis. However, in the present study, the urinary purine derivative excretions were not affected by dietary treatment, a result which did not confirm a linear increase in microbial protein yield as the proportion of BR was increased in the diet. On the other hand, Ohtani et al. (2001) suggested that increasing the fermentable starch in the diet could increase the level of ruminal propionic acid, and then decrease urinary N losses by decreasing the use of amino acids for gluconeogenesis. Additionally, Lemosquet et al. (2004) reported that the use of some amino acids for gluconeogenesis increases when the dietary supply of starch is low. In the present study, the starch digestibility, glucogenic material such as ruminal propionate, and plasma glucose concentration increased as the level of BR rose. Increases such as that of rumen propionate suggest that more glucose precursors could be supplied, and supplying more glucose precursors might inhibit hepatic gluconeogenesis by amino acid deamination. Thus, an increased level of BR in the diet might increase N retention and decrease urinary N losses by decreasing deamination.

CONCLUSIONS

The substitution of BR for corn resulted in DMI depressions and decreased milk production of cows. The

present results are inconsistent with our previous findings, which reported that steam-flaked BR was used as a replacement for steam-flaked corn grain included in rations to a level of at least 30% of dietary DM without compromising milk production. The effect of replacing corn with BR on milk production is dependent on the grain proportion in diets. When cows were fed diets with a high proportion of grain at 40% of dietary DM, substituting BR for corn caused adverse effects on the productivity of lactating cows. As a proportion of N intake, urinary N excretion decreased and N retention increased with the increasing BR. This is consistent with our previous reports showing that substituting BR for corn decreased the urinary N losses and improved N utilization.

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