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Digestibility, lactation performance, plasma metabolites, ruminal fermentation, and bacterial communities in Holstein cows fed a fermented corn gluten-wheat bran mixture as a substitute for soybean meal

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ABSTRACT

The purpose of this research was to investigate the effects of replacing soybean meal (SBM) with a fermented corn gluten-wheat bran mixture (FCWM) on nutrient digestibility, lactation performance, plasma metabolites, ruminal fermentation, and bacterial communities in Holstein cows. Nine healthy multiparous (parity = 3) Holstein cows with similar body weights (624 ± 14.4 kg), days in milk (112 ± 4.2), and milk yields (31.8 ± 1.73 kg; all mean ± standard deviation) were used in a replicated 3 × 3 Latin square design with 3 periods of 28 d. Cows were fed 1 of 3 dietary treatments in which FCWM replaced SBM as follows: basal diet with no replacement (0FCWM); 50% replacement of SBM with FCWM (50%FCWM); and 100% replacement of SBM with FCWM (100%FCWM). The diets were formulated to be isocaloric and isonitrogenous. The results showed that the total-tract digestibility of dry matter and crude protein increased linearly with increased dietary FCWM, and we found a trend for increased total-tract neutral detergent fiber and potentially digestible NDF digestibility. Milk yield tended to increase in a linear manner as more FCWM was consumed, and energy-corrected milk production was significantly increased with FCWM supplementation as a result of increased milk protein and lactose yields. Plasma glucose and IgG concentrations increased linearly with increasing FCWM supplementation, but plasma malondialdehyde concentration decreased linearly. Concentrations of total volatile fatty acids and propionate showed a linear increase with increasing FCWM supplementation, leading to a linear decrease in pH. The relative abundance of ruminal *Prevotellaceae*, *Veillonellaceae,* and *Prevotella* 1 increased linearly with increasing FCWM supplementation, and the relative abundance of ruminal *Succinivibrionaceae* and *Muribaculaceae* decreased linearly. The relative abundance of fecal *Ruminococcaceae*, *Prevotellaceae,* and *Ruminococcaceae* UCG-005 increased linearly with increasing FCWM supplementation, but the relative abundance of fecal *Peptostreptococcaceae* decreased linearly. Overall, the replacement of SBM with FCWM altered the composition of the ruminal bacterial community and improved nutrient digestibility, lactation performance, and ruminal fermentation in cows, providing a data reference for the use of FCWM in dairy production.

Key words: fermented corn gluten-wheat bran mixture, dairy cow, performance

INTRODUCTION

In China, the demand for high-quality protein feed is gradually increasing with the continuous development of the dairy industry. Soybean meal (**SBM**), as a traditional ingredient, has always been the most widely used protein supplement in ruminant diets (Silva et al., 2015). However, few byproducts with high-quality protein are available in China other than SBM, making the high-quality protein feed supply insufficient and leading to the need for imports. This not only increases the feed costs incurred by dairy farms but also makes the Chinese dairy industry less competitive in the global market. For these reasons, the search for resource-rich high-quality protein supplements for cows is crucial for promoting the development of the Chinese dairy industry.

Corn is one of the most widely distributed food crops in the world and plays an important role in agricultural production. In China, one of the main uses of corn is to produce starch, and a large number of byproducts are generated from starch processing. Among these byproducts is corn gluten meal, which has an annual output of over 2 × 10^6 t, high protein content, and no antinutritional factors (Regost et al., 1999). Nevertheless, previously published literature has shown that corn gluten meal is difficult to use as a major protein ingredient in the diets of dairy cows (Wohlt et al., 1991; Holter et al., 1992; Cozzi and Polan, 1994). The

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reasons for its limited use include its unpleasant taste, low biological value, and lack of essential amino acids (Turk et al., 1935; Wu, 2004). Interestingly, current research in fermentation science has demonstrated ways to improve the nutritional value of byproduct feeds by improving their digestibility, amino acid profile, and flavor (Zhang et al., 2007; Kim et al., 2012). Lactic acid bacteria (**LAB**), a class of gram-positive bacteria, are widely used in feed fermentation because they can inhibit the growth of harmful bacteria and improve the palatability of feed (Soukoulis et al., 2007; Reich and Kung, 2010). A previous study by our research team showed that wheat bran is an inexpensive feed ingredient and, when combined with corn gluten meal, could provide a reasonable source of digestible nutrients for fermentation (Wang et al., 2019). Furthermore, the addition of protease is beneficial in improving the biological value of corn gluten-wheat bran mixture (**CWM**) by producing bioactive peptides (Zhou et al., 2015a). Consequently, we used LAB and acid protease in the present study to ferment CWM.

Over the years, a few published studies have demonstrated that ruminants fed fermented products showed improved performance in terms of increased antioxidant and immune capacity, improved ruminal fermentation, and alterations in the composition of the rumen and hindgut bacterial community (Fujisawa et al., 2010; Rodríguez-Muela et al., 2015; Guo et al., 2019). Similarly, our recent study found that fermented corn gluten meal could be used in calf diets to improve antioxidant and immune capacity and alter the composition of the rumen and fecal bacterial community, thereby promoting the absorption of nutrients and leading to higher growth performance (Jiang et al., 2020). Nevertheless, the effects of replacing SBM with fermented corn gluten-wheat bran mixture (**FCWM**) on dairy cows are still unknown. Our hypothesis for this study was that FCWM may be used in the diets of dairy cows to replace SBM and improve their production. The purpose of the current research was to investigate whether the use of FCWM as a substitute for SBM would have positive effects on nutrient digestibility, lactation performance, plasma metabolites, ruminal fermentation, and bacterial communities in Holstein cows.

MATERIALS AND METHODS

Preparation of FCWM

The LAB (*Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC BAA-365 and *Lactobacillus acidophilus* ACCC 10637; 1:1) were provided by Xinlaiwang Biotechnology Co. Ltd. (Nanjing, China). Corn gluten meal and wheat bran were provided by Harbin Sanhe Feed Co. Ltd. (Harbin, China). Acid protease (50,000 U/g), which has the ability to effectively hydrolyze the protein in feed, was purchased from Beijing Baileibo Technology Co. Ltd. (Beijing, China); the protease can be used effectively at a temperature of 30°C to 50°C and a pH of 2.5 to 6.0. Fermentation was carried out by Harbin Prosyn Microbial Technology Co. Ltd. (Harbin, China). A total of 600 kg of CWM, consisting of 360 kg of corn gluten meal and 240 kg of wheat bran, was put into a fermentation tank (Nanjing Runzhe Biology Engineering Facility Co. Ltd., Nanjing, China) with a regulated temperature, and the corn gluten meal and wheat bran were mixed evenly by stirring. Then, LAB (5×10^9 cfu/g) and acid protease (5 g/kg) were added to 50 L of water and stirred until the enzyme was dissolved. To ensure that the CWM contacted the LAB and acid protease evenly, the solution was added to the fermentation tank in the form of a spray, and stirring and addition were carried out at the same time. After adding the solution, the remaining water was slowly added to the tank to adjust the moisture content of the CWM to approximately 40%. The mixture was stirred continuously until it was mixed evenly. Then, the fermenter was sealed tightly, the temperature was adjusted to 36°C, and fermentation was carried out for 96 h to obtain FCWM. After fermentation, the mixture was transferred to 28 fermentation bags (62 cm × 105 cm) with a 1-way breather valve, and the bags were vacuum-sealed. The FCWM was then transported to the dairy farm by truck (1.5 h journey) and stored in a cool warehouse for the feeding experiment. The CWM was fermented in 3 batches and used for the 3 periods of 28 d each.

Animals, Experimental Design, and Diets

The experiment was performed under an experimental license from Northeast Agricultural University, (Harbin, China). This experiment was conducted at Wandashan Dairy Farm Harbin, China, from June to August 2019, and all experimental procedures involving animals were performed in accordance with the principles and responsibilities outlined in the university's guidelines for animal research. Nine healthy multiparous (parity = 3) Holstein cows with similar BW (624 ± 14.4 kg), DIM (112 ± 4.2), and milk yield (31.8 ± 1.73 kg; all mean ± standard deviation) were enrolled in a replicated 3 × 3 Latin square design with 3 periods of 28 d. In each period, the initial 21 d were used for diet adaption, and the final 7 d were used for sample collection. Cows were fed 1 of 3 dietary treatments, in which FCWM replaced some portion of SBM as follows: basal diet without replacement (0FCWM); 50% replacement of SBM with FCWM (50%FCWM); and

Table 1. Ingredients and chemical composition (% of DM) of diets containing different amounts of fermented corn gluten-wheat bran mixture (FWCM)[1]

Item	0FCWM	50%FCWM	100%FCWM
Ingredient			
Corn silage	22.1	22.1	22.1
Oat hay	17.1	17.1	17.1
Alfalfa hay	11.2	11.2	11.2
Corn grain, steam rolled	20.6	20.0	19.4
Soybean meal	14.0	7.00	0.00
FCWM	0.00	7.60	15.2
Whole cottonseed	5.88	5.88	5.88
Sunflower meal	3.52	3.52	3.52
Beet pulp	4.10	4.10	4.10
Premix[2]	1.50	1.50	1.50
Chemical composition			
CP	16.7	16.6	16.5
NDF	35.5	35.4	35.3
ADF	20.8	20.6	20.1
NFC	38.7	39.1	39.4
Ether extract	3.80	3.90	3.99
Starch	22.1	22.1	22.4
Lys, % requirement	115	108	98
Met, % requirement	119	120	124
NE_L,[3] Mcal/kg of DM	1.64	1.63	1.63
ME for milk,[4] kg/d	40.2	40.0	39.6
MP for milk,[5] kg/d	41.4	41.7	41.8
Rumen N balance			
Peptide, g/d	72.0	64.0	59.0
Peptide, % requirement	130	127	125
Peptide and NH_3, g/d	79.0	61.0	45.0
Peptide and NH_3, % requirement	118	114	111

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with FCWM; 100%FCWM = 100% replacement of soybean meal with FCWM.

[2]Contained per kilogram of premix: Ca 142.5 g, P 54.0 g, Mg 49.3 g, Na 106.4 g, Cl 29.5 g, K 500 mg, S 3.7 g, Co 12 mg, Cu 500 mg, Fe 4.858 g, I 25 mg, Mn 800 mg, Se 10 mg, Zn 1.8 g, vitamin A 180,000 IU, vitamin D 55,000 IU, and vitamin E 1,500 IU.

[3]Calculated according to the Ministry of Agriculture of P.R. China (China NY/t34, 2004).

[4]Metabolizable energy allowable milk yield predictions from CPM Dairy (Tedeschi et al., 2008).

[5]Metabolizable protein allowable milk yield predictions from CPM Dairy (Tedeschi et al., 2008).

100% replacement of SBM with FCWM (100%FCWM). The isocaloric and isonitrogenous diets (Table 1) were formulated using the Cornell-Penn-Miner dairy model (CPM Dairy, version 3.0.10; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to meet the nutrient requirements of a 650-kg cow producing 39.0 kg/d milk with 3.90% fat and 3.25% protein (Tedeschi et al., 2008). Throughout the study period, the cows were housed in individual freestall pens (4 m × 5 m) with concrete floors and clean rice husk bedding (refreshed daily), equipped with self-locking head gates at the feed line. Cows were milked twice daily (0600 to 0630 h and 1800 to 1830 h) in a 40-stand DeLaval rotary milking parlor (DeLaval International, Tumba, Sweden). The TMR was weighed and fed to cows twice per day, at 0630 h and 1830 h, immediately after cows were milked. Refusals from each day were collected every morning before feeding and weighed. Based on the feed intake of the cow the day before, the amount of feed offered was adjusted daily to allow for at least 5% refusal (on an as-fed basis). The feed was pushed up at least 10 times per day. Cows had free access to fresh water throughout the study period.

Data Collection and Sampling

Feeds, Feces, and Milk. The TMR offered and refused for individual cows was weighed for 7 consecutive days (d 22 to 28 of each period) to calculate DMI. Samples of individual feed ingredients, orts, and TMR were collected during the final 3 d of each period, stored at −20°C, and later pooled by cow and by period. Thereafter, all feed samples were dried at 55°C for 48 h, milled to pass through a 1-mm screen, and stored in sealed plastic bags (150 mm × 220 mm) at 4°C until determination of chemical composition. On d 26, 27, and 28 of each period, approximately 500-g spot fecal samples were taken from the rectum at 0600 and 1800 h, and samples were composited for each cow. The collected fecal samples were dried at 55°C for 48 h, milled to pass through a 1-mm screen and

stored in sealed plastic bags at 4°C until determination of nutrient digestibility. As well, a portion of the fecal samples (approximately 5 g) collected at d 28 was stored instantly in liquid nitrogen until determination of bacterial communities. Milk production was recorded for 7 consecutive days (d 22 to 28 of each period). On d 26, 27, and 28 of each period, milk samples for each cow were collected at each milking, and a 24-h composite milk sample (50 mL) was prepared according to the actual proportion of milk yield at each milking. Milk samples were mixed with potassium dichromate and stored at 4°C until determination of milk composition.

Blood, Ruminal Fluid, and Urine. Blood was collected from the coccygeal vein in sodium heparin tubes 3 h after the morning feeding on 3 consecutive days (d 26, 27, and 28 of each period). Then, blood samples were centrifuged at 2,000 × *g* for 15 min at 4°C to obtain plasma, and stored at −20°C until determination of plasma indices. Three hours after the morning feeding on d 26 to 28 of each period, ruminal fluid samples were collected via an oral stomach tube equipped with a vacuum pump. We discarded the first 100 to 200 mL of fluid collected to reduce the chance that the stomach-tube rumen samples were contaminated with saliva. The ruminal fluid samples were strained through 4 layers of cheesecloth, and pH was measured immediately using a pH meter (PHS-3C; Nanjing Nanda Analytical Instrument Application Research Institute, Nanjing, China). Then, a 10-mL sample was acidified by mixing with 2 mL metaphosphoric acid (25%, wt/vol), and the mixture was centrifuged at 3,000 × *g* for 15 min. The supernatant was separated and stored at −20°C until determination of VFA. Another 10-mL sample was acidified by mixing with 0.2 mL sulfuric acid (50%, vol/vol) and stored at −20°C until determination of ammonia-N levels. Urine samples were collected for all cows during urination with stimulation at 0600 and 1800 h on d 26, 27, and 28 of each period. The 10-mL urine sample collected was acidified immediately by mixing with 40 mL of H_2SO_4 (0.036 mol/L) and stored at −20°C for future estimation of microbial protein (**MCP**) yield. Before starting determination, urine samples were thawed and composited (10 mL) by volume for each cow in each period.

Laboratory Analysis

Nutritional Composition of Feeds. Samples of individual feed ingredients, orts, and TMR were sent to the Animal Nutrition Laboratory of Northeast Agricultural University (Harbin, China) for nutrient analysis using wet chemistry methods. The DM, ash, CP, and ether extract contents of the samples were determined according to the procedures of AOAC International (2000). Contents of heat-stable α-amylase-treated NDF and ADF were analyzed according to a previously reported method (Van Soest et al., 1991). Starch content was measured using the Megazyme Total Starch Assay Kit (product no: K-TSTA; Megazyme International Ireland Ltd., Wicklow, Ireland). Samples of the CWM, FCWM, SBM, and TMR were also sent to the Heilongjiang Academy of Agricultural Sciences (Harbin, China) for analysis of amino acid composition using a fully automatic amino acid analyzer (S-433D; Secam Scientific Instrument Co., Ltd., Beijing, China) according to the GB/T 18246–2000 method (GB China National Standards, 2000). Briefly, sulfur amino acids (Met and Cys) were measured after oxidation of the samples with performic acid at 0°C for 16 h; Trp was measured after hydrolyzation of the samples with lithium hydroxide solution (4 mol/L) at 110°C for 20 h; and the other amino acids were measured after hydrolyzation of the samples with 6 mol/L HCl at 110°C for 24 h. The small peptide contents of the CWM, FCWM, and SBM were measured using the trichloroacetic acid method described by Iemura et al. (1999). The total phenol contents of the CWM, FCWM, and SBM were detected using the Folin–Ciocalteu reagent colorimetric method reported by Kim et al. (2006).

Nutrient Digestibility and Milk Composition. Total-tract apparent nutrient digestibility was estimated using the concentration of indigestible NDF (**iNDF**) in the diet and feces as an internal marker, as reported by Lee and Hristov (2013). The iNDF content in the feces, TMR, and orts was determined by in situ incubation for 288 h, as detailed by Huhtanen et al. (1994). The DM, CP, NDF, and ADF contents of fecal samples were analyzed using the procedures described for the feeds. The digestibility of DM (%) was calculated as [1 – (% of iNDF intake/% of iNDF in feces)] × 100%. The digestibility of nutrients (%) was calculated as {1 – [(% of iNDF intake/% of iNDF in feces) × (% of nutrient in feces/% of nutrient intake)]} × 100%. Milk samples were sent to the Heilongjiang Academy of Agricultural Reclamation (Harbin, China) for analysis of protein, fat, lactose, and MUN concentrations, as well as SCC, using a 4-channel spectrophotometer (MilkoScan; Foss Electric, Hillerød, Denmark).

Plasma, Ruminal Fermentation, and MCP Synthesis. Plasma glucose concentration was measured using a fully automatic biochemical analyzer (HT82-BTS-330; Xihuayi Technology Co. Ltd., Beijing, China). A commercial thiobarbituric acid kit from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China) was used to analyze plasma malondialdehyde concentration. Plasma IgG concentration was determined using commercial ELISA kits (SINO-UK Institute of Biotechnology, Beijing, China). Concentra-

tions of plasma triglycerides, cholesterol, nonesterified fatty acids, urea, glucose, BHB, total superoxide dismutase, total antioxidant capacity, catalase, glutathione peroxidase, soluble CD3, soluble CD4, IgA, IgM, triiodothyronine, thyroxine and prolactin were also measured, but the differences in these concentrations among treatments were not significant ($P > 0.10$). The methods and results for these measurements can be found in Supplemental Table S1 (https://doi.org/10.7910/DVN/8IVPT0). Concentrations of VFA were determined by gas chromatography (GC-8A; Shimadzu Corp., Kyoto, Japan; Stewart and Duncan, 1985). Concentrations of ammonia-N were determined using the phenol/hypochlorite method (Broderick and Kang, 1980). Yield of MCP was calculated according to the estimates of urinary purine derivative (allantoin and uric acid) excretion (Chen and Gomes, 1992). Valadares et al. (1999) and Leonardi et al. (2003) have demonstrated that creatinine can be used as a marker to estimate urine volume. Allantoin and uric acid concentrations were detected based on the method described by Chen and Gomes (1992), and creatinine was detected by a colorimetric picric acid method (Shingfield and Offer, 1999). In calculating urine volume, creatinine output was assumed to average 28 mg/kg of BW, as estimated by Valadares et al. (1999).

Bacterial Communities. Bacterial community analysis of rumen and fecal samples was carried out at Biomarker Technologies Co., Ltd., (Beijing, China) using high-throughput sequencing. The DNA was extracted from the samples using an MN NucleoSpin 96 Soi DNA kit (Gene Company Limited, Beijing, China) in accordance with the kit's instructions. The DNA obtained from each sample was subjected to 2-step PCR amplification to construct a small-fragment sequencing library. For the first amplification step, the 16S rRNA gene spanning V3 to V4 (primers: 338F, 5′-ACTCCTACGGGAGGCAGCA-3′; 806R, 5′-GGACTACHVGGGTWTCTAAT-3′) was amplified using the extracted DNA as a template. The PCR was carried out using a Veriti 96-well PCR instrument (9902; Applied Biosystems Inc., Foster City, CA) in a 10-μL reaction as follows: 50 ng of genomic DNA, 0.3 μL of Vn F (10 μmol/L), 0.3 μL of Vn R (10 μmol/L), 5 μL of KOD FX Neo Buffer, 2 μL of dNTP (2 mmol/L), 0.2 μL of KOD FX Neo, and 2.2 μL of distilled, deionized H_2O. The PCR conditions were as follows: 1 cycle at 95°C for 5 min; 25 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 40 s; and a final extension of 72°C for 7 min. Then, the PCR products obtained in the first-step amplification were used as templates for the second-step Solexa PCR amplification (Applied Biosystems Inc.). The Solexa PCR amplification system of 20 μL contained the following: 5 μL of target region PCR purified product, 2.5 μL of MPPI-a (2 μmol/L), 2.5 μL of MPPI-b (2 μmol/L), and 10 μL of 2 × Q5 HF MM. The Solexa PCR conditions were as follows: 1 cycle of 98°C for 30 s; 25 cycles of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s; and a final extension of 72°C for 5 min. The Solexa PCR products were recycled from a 1.8% agarose gel and purified using an OMEGA DNA purification column (Gene Company Limited). The purified products were quantified using a Quant-iT PicoGreen dsDNA Assay Kit (Gene Company Limited) in accordance with the kit's instructions. Then, the amplicons were sequenced on an Illumina HiSeq 2500 sequencing platform (Illumina Inc., San Diego, CA) using the paired-end sequencing method. The original sequences obtained (2,159,887 and 2,137,782 reads for the ruminal and fecal samples, respectively) were spliced using FLASH software (version 1.2.11; Magoč and Salzberg, 2011) to obtain the original tag data (2,114,198 and 2,085,430 raw tags for the ruminal and fecal samples, respectively). The raw tags obtained were filtered using Trimmomatic software (version 0.33; Bolger et al., 2014) to obtain high-quality clean tag data (2,094,696 and 2,065,664 clean tags for the ruminal and fecal samples, respectively). We then used UCHIME software (version 8.1; Edgar et al., 2011) to identify and remove chimeric sequences to obtain effective tags (1,968,567 and 1,915,609 effective tags for the ruminal and fecal samples, respectively). The tags were binned into operational taxonomic units (**OTU**) using the clustering program USEARCH (version 10.0; Edgar, 2013) based on a 97% sequence similarity level. The OTU obtained were eventually used for taxonomic assignment. The representative sequences for each OTU were compared with the Silva (Release 128; www.arb-silva.de) database for obtaining taxonomic classification at the phylum, class, order, family, and genus levels. A rarefaction curve was constructed to ensure that sufficient sequencing depth had been achieved. The relative abundances of taxa at the family and genus levels were determined using QIIME software (Kuczynski et al., 2011) to compare the bacterial community composition in the rumen and feces among different dietary treatments. Richness and diversity indices were also determined using MOTHUR software (version 1.30; Schloss et al., 2009) to compare bacterial diversity among the dietary treatments. The raw sequences data generated from this study have been submitted to the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA669464.

Statistical Analysis

Before analyses, carryover effects were determined using the general linear model in Minitab (version 17.0;

Pennsylvania State University, State College, PA); no carryover effects were detected ($P > 0.05$) for any of the data. Therefore, all data from the experiment were analyzed in a 3 × 3 Latin square design using the Proc Mixed procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC), according to the model $Y_{ijkm} = \mu + T_i + P_j + C_k + S_m + E_{ijkm}$, where Y_{ijkm} was the observation, μ was the overall mean, T_i was the fixed effect of the treatment, P_j was the fixed effect of the period, C_k was the random effect of the cows, S_m was the fixed effect of the square, and E_{ijkm} was the residual error. Orthogonal polynomial contrasts were also used to analyze the linear and quadratic effects of increasing FCWM supplementation on each variable. Duncan's multiple range tests were used for this experiment. Significant differences were declared at $P \leq 0.05$, and trends were defined at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

The ingredients and chemical composition (% DM) of the 3 experimental diets are shown in Table 1. To ensure the isoenergetic (ME for milk) and isonitrogenous (MP for milk) characteristics of the 3 diets, the concentration of corn grain in the diets was slightly decreased as FCWM concentration was increased. The concentrations of NDF, ADF, ether extract, and starch were also similar in the 3 diets. The chemical composition (%DM) of the SBM, CWM, and FCWM is presented in Table 2. The DM content of FCWM was the lowest among the 3 feeds; the higher moisture content of the FCWM renders it less suitable for long-term preservation, which may limit its use. The fermentation process increased the contents of ether extract, CP, and small peptide contents in the CWM and decreased the contents of DM, NDF, ADF, and starch. The increase in CP content might have been due to the decomposition of carbohydrates in the CWM into carbon dioxide gas via LAB during the fermentation process, reducing the total amount of carbohydrates such as NDF, ADF, and starch, and increasing the relative content of protein. The increase in small peptide content could be attributed to the degradation of large-molecule proteins in the CWM into small-molecule proteins via acid protease during fermentation. We also observed that the total phenol content in the FCWM was higher than in the CWM, which may have been due to the release of phenolic compounds by hydrolyzing the CWM under acidic conditions (Kim et al., 2006). This finding was in accordance with previously reported results (Zhao et al., 2017), in which fermentation with LAB improved the total phenol content in wheat bran. The LAB most likely affected the digestibility of the wheat bran, resulting in the release of more phenols. These phenolics are likely related to Klason lignin, which partially represents the nonpolymerized phenolics in the plant structure. For amino acid composition, an interesting finding was that the Lys content in the FCWM was higher than that in the CWM (1.27 vs. 0.65%). This phenomenon may have been related to the use of *Lactobacillus delbrueckii* ssp. *bulgaricus*, which was selected as an inoculum to ferment the CWM and has shown the ability to produce Lys during fermentation (Luo et al., 2010). It is likely that the Lys comes from the microbial mass that has accumulated after fermentation. These results suggested that the nutritional quality of the CWM was improved after fermentation, elevating the possibility of its use as a substitute for SBM in the diets of dairy cows. The FCWM had lower concentrations of CP and ADF than the SBM, but higher concentrations of ether extract and starch . It is well known that Met and Lys are the most limiting amino acids for milk production (Schwab et al., 1992). The Lys content in the FCWM was lower than that in the SBM (1.27 vs. 2.51%), which may result in a slight decrease in milk protein production (Kleinschmit et al., 2006). However, our findings showed that the milk protein content was increased in FCWM-fed cows (Table 3). This may have been linked to higher Met content (FCWM vs. SBM, 1.18 vs. 0.68%), improved ruminal fermentation, and increased MCP synthesis, as well as nutrient digestibility. Mjoun et al. (2010) demonstrated that the replacement of SBM with dried distillers grains with solubles (containing 1.09% Lys, DM basis) boosted the milk protein content of cows.

Lactation Performance

As presented in Table 3, cows fed 50%FCWM and 100%FCWM showed linearly increased ECM ($P = 0.04$), protein ($P = 0.001$), and lactose ($P = 0.01$) yields, and tended to show linearly increased milk yield ($P = 0.09$) than cows fed 0FCWM. Moreover, concentrations of milk protein ($P = 0.01$) and lactose ($P = 0.04$) also showed a linear increase. We observed no differences among treatments ($P > 0.10$) in DMI, 4% FCM yield, fat yield, fat percentage, MUN concentration, SCC, or feed efficiency. Increased milk yield might have been a result of the enhanced nutrient digestibility observed in dairy cows fed the FCWM diets. Increased ruminal concentrations of propionate with the FCWM diets (Table 4) is another potential reason for the increased milk yield, because propionate is a glucogenic precursor; increasing glucogenic precursors

Table 2. Chemical composition of soybean meal (SBM), corn gluten-wheat bran mixture (CWM), and fermented CWM (FCWM)

Item	SBM	CWM	FCWM
Proximate composition			
DM, %	88.7	89.1	58.6
Ash, % of DM	5.98	2.52	3.61
CP, % of DM	48.0	42.2	44.3
Ether extract, % of DM	2.11	2.10	3.17
NDF, % of DM	17.0	21.7	17.6
ADF, % of DM	10.4	7.96	6.21
Starch, % of DM	3.69	12.1	9.01
Small peptide, % of DM	5.33	2.87	13.2
Total phenol, mg/g of DM	3.06	3.64	9.42
AA composition, % of DM[1]			
Arg	3.43	1.37	1.52
His	1.22	0.80	0.86
Ile	2.10	1.89	1.92
Leu	4.11	7.73	8.01
Lys	2.51	0.65	1.27
Met	0.68	1.14	1.18
Phe	2.63	3.53	3.77
Trp	0.65	0.31	0.37
Thr	1.85	1.32	1.34
Val	2.26	2.13	2.10
Asp	4.98	2.37	2.25
Ala	2.21	2.90	3.08
Glu	7.74	7.63	7.49
Gly	1.85	1.30	1.41
Cys	0.73	0.62	0.65
Tyr	1.57	1.42	1.39
Ser	2.15	1.93	2.02
Pro	1.80	2.25	2.09

[1]The approximate composition was analyzed using wet chemistry methods.

[2]The AA composition was analyzed according to the method of GB/T 18246–2000 (China NY/t34, 2004), the oxidation (performic acid) hydrolysis method for sulfur AA, the alkaline (lithium hydroxide) hydrolysis method for Trp, and the acid (hydrochloric acid) hydrolysis method for the other AA.

resulted in an increase in blood glucose availability via gluconeogenesis and thereafter in increased milk yield and lactose production, as well as lactose concentration in milk (Sauer et al., 1989; Stein et al., 2006). Previously published literature has demonstrated that increased ruminal concentrations of propionate boosted milk yield and protein concentration in milk (Rigout et al., 2003). In addition, milk protein secretion is closely related to MCP, which is a vital protein source for ruminants, accounting for approximately 40 to 80% of the total intestinal protein (Wang et al., 2018). Available evidence has found that increased MCP production contributed to increased milk production and increased milk protein concentration (Zhou et al., 2015b). Consequently, the linear increase in the MCP yield of cows fed 50%FCWM and 100%FCWM (Table 4) in the present study may be another potential reason for the enhanced milk yield and milk protein concentration.

Ruminal Fermentation and MCP Synthesis

We observed no difference ($P > 0.10$) in the concentrations of ammonia-N, acetate, butyrate, isovalerate, or valerate with FCWM supplementation (Table 4). However, the ammonia-N concentration was relatively high by current standards, which may cause a small amount of nitrogen to be wasted. Ruminal pH ($P = 0.01$) showed a linear decrease with FCWM supplementation, suggesting that the ruminal fermentation pattern was changed after FCWM was fed to cows. Indeed, the concentrations of total VFA ($P = 0.02$) and propionate ($P = 0.003$) in cows fed 50%FCWM

Table 3. Milk production, milk composition, and feed efficiency in Holstein cows fed 3 diets

	Treatment[1]				*P*-value	
Item	0FCWM	50%FCWM	100%FCWM	SEM	Linear	Quadratic
Production, kg/d						
Milk	32.3	33.8	34.2	1.09	0.09	0.62
ECM[2]	32.8	37.1	36.6	1.34	0.04	0.13
4% FCM[3]	29.6	33.2	32.3	1.38	0.17	0.16
Fat	1.11	1.32	1.24	0.08	0.27	0.16
Protein	0.99	1.12	1.18	0.04	0.001	0.39
Lactose	1.57	1.71	1.77	0.06	0.01	0.60
Composition						
Fat, %	3.44	3.89	3.62	0.22	0.56	0.21
Protein, %	3.05	3.34	3.45	0.10	0.01	0.47
Lactose, %	4.86	5.06	5.18	0.11	0.04	0.77
MUN, mg/dL	13.9	13.5	14.8	1.18	0.69	0.26
SCC, $\times 10^3$/mL	115.3	104.6	100.7	9.48	0.24	0.74
DMI, kg/d	21.7	21.8	22.9	1.06	0.20	0.53
Feed efficiency						
Milk/DMI	1.51	1.55	1.49	0.07	0.86	0.45

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

[2]ECM = 0.3246 × milk yield + 13.86 × milk fat yield + 7.04 × milk protein yield (Orth, 1992).

[3]4% FCM = 0.4 × milk yield + 15 × fat yield.

Table 4. Ruminal fermentation and microbial protein synthesis in Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
pH	6.56	6.32	6.29	0.06	0.01	0.17
Ammonia-N, mg/dL	17.8	16.6	18.6	1.21	0.44	0.11
Total VFA, mmol/L	88.5	94.0	99.2	3.04	0.02	0.96
Acetate, mmol/L	55.7	58.1	60.1	1.97	0.13	0.91
Propionate, mmol/L	21.1	24.2	26.1	1.08	0.003	0.64
Butyrate, mmol/L	10.5	10.4	11.9	0.65	0.15	0.36
Isovalerate, mmol/L	0.71	0.71	0.67	0.03	0.38	0.59
Valerate, mmol/L	0.51	0.54	0.56	0.03	0.11	0.92
Acetate:propionate	2.67	2.42	2.33	0.10	0.02	0.49
Microbial protein, kg/d	1.65	1.70	1.84	0.06	0.01	0.46

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

and 100%FCWM showed a linear increase compared to the cows fed 0FCWM, and this may explain why the pH was decreased. The decreased pH, altered VFA profile, and decreased ratio of milk fat to protein may have increased the risk of acidosis in cows fed FCWM compared to cows fed SBM. The increased propionate concentration and slightly increased acetate concentration resulted in a linear decrease in the ratio of acetate to propionate ($P = 0.02$). It is likely that the fermentation process improved the rumen fermentability of the FCWM, which resulted in increased action of the remaining carbohydrates in that diet, thereby increasing the total VFA and propionate concentrations. It is also possible that the increased digestibility of DM, CP, and NDF in the FCWM-fed cows improved readily fermentable carbohydrate ingestion and led to higher VFA concentrations (Khorasani et al., 2001). In line with our results, previously published literature has reported that calves fed fermented corn gluten meal after weaning showed increased ruminal VFA concentrations (Jiang et al., 2019). We also observed that the MCP yield ($P = 0.01$) showed a linear increase with FCWM supplementation. The increased VFA concentrations and digestibility of CP may also have provided more energy and available nitrogen for microbial growth and, ultimately, allowed MCP synthesis to increase. The improved ruminal fermentation patterns and increased MCP production may play an important role in boosting the lactation performance of dairy cows.

Plasma Metabolites

As presented in Table 5, plasma glucose concentration ($P = 0.01$) showed a linear increase with FCWM supplementation. This increase in glucose concentration could be attributed to increased concentrations of propionate, which is a precursor for glycogenesis and can promote blood glucose synthesis via gluconeogenesis (Sauer et al., 1989). An increase in glucose concentration is beneficial for the maintenance of normal physiological functions and increases milk yield in dairy cattle (Stein et al., 2006). Malondialdehyde is one of the most important products of membrane lipid peroxidation, and its production can also aggravate membrane damage (Barrera et al., 2018). In this study, malondialdehyde ($P = 0.01$) concentration in plasma showed a linear decrease when the cows were fed increasing amounts of FCWM, indicating that the accumulation of lipid peroxidation products was slowed in cows fed FCWMs. A possible cause for the reduced malondialdehyde concentration may have been the higher total phenolic content in FCWM compared to SBM. Gobert et al. (2009) and Santos et al. (2016) demonstrated that phenolic compounds can reduce the accumulation of lipid peroxidation products in blood by capturing radicals, bonding with metal ions, or both. The plasma IgG concentration ($P = 0.03$) in 50%FCWM and 100%FCWM increased linearly compared to 0FCWM, suggesting that the addition of FCWM to the diet might improve the humoral immunity of dairy cows. This finding was similar to previously published results (Jiang et al., 2019), in which calves fed fermented corn gluten meal after weaning showed improved plasma IgG concentrations.

Intake and Total-Tract Apparent Digestibility

The results for intake and total-tract apparent digestibility are presented in Table 6. Intakes of CP, NDF, potentially digestible NDF and ADF were not significantly different ($P > 0.10$) among treatments, although they were slightly increased with increasing FCWM supplementation. These results indicated that replacing FCWM with SBM did not affect the cow's willingness

Table 5. Plasma metabolites in Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
Glucose, mmol/L	3.32	3.49	3.74	0.12	0.01	0.79
Malondialdehyde, nmol/mL	5.41	4.45	4.02	0.44	0.01	0.53
IgG, g/L	8.46	9.56	10.1	0.66	0.03	0.59

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

to eat. This could be attributed to improvements in the acidic taste of CWM with fermentation, which could mask the bitterness of the CWM. The total-tract apparent digestibility of ADF was not affected ($P > 0.10$) by FCWM treatment. However, cows fed 50%FCWM and 100%FCWM not only showed a linearly increased digestibility of DM ($P = 0.03$) and CP ($P = 0.02$); they also tended to showed a linearly increased digestibility of NDF ($P = 0.06$) and potentially digestible NDF ($P = 0.08$) compared to cows fed 0FCWM. This finding may have been related to changes in the ruminal and intestinal bacterial communities of cows fed different diets. Boone et al. (2011) demonstrated that *Prevotellaceae* is mainly responsible for the degradation of protein, amino acids, and starch in diets, promoting the metabolism of protein in cows. A previous study reported that *Ruminococcaceae* plays an important role in fiber metabolism, so a higher relative abundance of *Ruminococcaceae* results in an increase in dietary fiber digestion (Patra and Yu, 2015). In the present study, we observed a linear increase in the relative abundance of *Prevotellaceae* in the ruminal fluids and feces and a linear increase in the relative abundance of *Ruminococcaceae* in the feces after feeding FCWM to cows (Table 7 and Table 8), which was a possible explanation for the linear increase in the digestibility of DM, CP, and NDF.

Bacterial Communities

Bacterial Community Composition. The clustering results showed that a total of 16 phyla, 24 classes, 32 orders, 54 families, 153 genera, and 171 species belonging to bacteria were identified in the ruminal samples. The results of the relative abundances (>0.5%) of the bacterial families and genera in the ruminal fluids are shown in Table 7. Among the identified families, the ruminal bacterial community composition in the present study was in accordance with known bacterial communities in dairy cows: bacteria from the *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* families appeared to dominate the core bacterial microbiome, regardless of dietary treatments, in line with other published literature (Thoetkiattikul et al., 2013; Zened et al., 2013). In addition, researchers also observed that ruminal bacterial communities contained a high relative abundance of *Flavobacteriaceae* (Thoetkiattikul et al., 2013). However, the relative abundance of *Flavobacteriaceae* in the present study was less than 0.5%, consistent with the results reported by Golder et al. (2014). For the bacterial family-level community composition among the different dietary treatments, we observed that the relative abundance of *Prevotellaceae* ($P = 0.001$) increased linearly with FCWM supplementation. This result agreed with previously published

Table 6. Intake and total-tract apparent digestibility of nutrients in Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
Intake, kg/d						
CP	3.62	3.62	3.78	0.19	0.13	0.64
NDF	7.70	7.72	8.08	0.44	0.23	0.69
Potentially digestible NDF	5.25	5.26	5.56	0.27	0.28	0.55
ADF	4.51	4.49	4.61	0.21	0.16	0.52
Digestibility, %						
DM	69.1	71.1	72.2	1.37	0.03	0.73
CP	71.2	74.3	75.5	1.78	0.02	0.55
NDF	46.0	47.8	49.1	1.52	0.06	0.85
Potentially digestible NDF	67.4	69.7	71.5	2.23	0.08	0.88
ADF	30.7	31.2	32.0	0.94	0.17	0.79

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

Table 7. Relative abundances (>0.5%) of the bacterial families and genera in the ruminal fluids of Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
Family						
Prevotellaceae	0.33	0.39	0.44	0.02	0.001	0.86
Ruminococcaceae	0.12	0.15	0.10	0.02	0.33	0.13
Lachnospiraceae	0.11	0.11	0.10	0.007	0.79	0.44
Acidaminococcaceae	0.07	0.09	0.09	0.01	0.46	0.65
Succinivibrionaceae	0.13	0.03	0.04	0.03	0.04	0.09
Muribaculaceae	0.07	0.04	0.03	0.006	0.002	0.16
F082	0.02	0.04	0.03	0.004	0.09	0.07
Rikenellaceae	0.02	0.03	0.03	0.003	0.06	0.15
Christensenellaceae	0.03	0.03	0.02	0.007	0.29	0.62
Veillonellaceae	0.02	0.02	0.04	0.005	0.02	0.29
Saccharimonadaceae	0.009	0.01	0.009	0.001	0.01	0.02
Others	0.08	0.07	0.06	0.007	0.29	0.72
Genus						
Prevotella 1	0.25	0.36	0.41	0.03	0.001	0.31
Succiniclasticum	0.07	0.09	0.09	0.01	0.45	0.65
Succinivibrionaceae UCG-001	0.11	0.02	0.01	0.03	0.03	0.19
Ruminococcus 2	0.04	0.06	0.03	0.01	0.78	0.11
Prevotella 7	0.07	0.01	0.02	0.02	0.04	0.15
Christensenellaceae R-7 group	0.03	0.03	0.02	0.007	0.29	0.62
Rikenellaceae RC9 gut group	0.02	0.03	0.03	0.002	0.06	0.14
Ruminococcaceae UCG-014	0.02	0.02	0.02	0.001	0.64	0.68
Succinivibrionaceae UCG-002	0.01	0.01	0.02	0.009	0.39	0.33
Lachnospiraceae NK3A20 group	0.02	0.02	0.01	0.002	0.08	0.35
Selenomonas 1	0.01	0.01	0.02	0.003	0.03	0.53
Candidatus Saccharimonas	0.008	0.01	0.02	0.004	0.01	0.35
Others	0.35	0.34	0.31	0.02	0.21	0.72

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

literature (Jiang et al., 2019), which demonstrated that the relative abundance of ruminal *Prevotellaceae* increased after fermented corn gluten meal was fed to calves. *Prevotellaceae* consists of gram-negative bacteria and has the ability to use a range of substrates, such as the CP, starch, and fiber in diets (Boone et al., 2011). Consequently, an increase in the relative abundance of *Prevotellaceae* may have improved the degradation of the dietary nutrients and contributed to improvements in lactation performance. *Veillonellaceae* ($P = 0.02$) increased linearly in relative abundance with increasing FCWM supplementation. The members of the *Veillonellaceae* are mainly responsible for propionate production by fermenting substrates (Strobel and Russell, 1991), in accordance with our finding of increased ruminal propionate concentration in the present study. *Rikenellaceae* ($P = 0.06$) increased linearly in relative abundance with increasing FCWM supplementation. *F082* ($P = 0.09$ and 0.07, respectively) and *Saccharimonadaceae* ($P = 0.01$ and 0.02, respectively) increased linearly and quadratically in relative abundance with increasing FCWM supplementation. These increased bacterial abundances might have led to a linear decrease in the relative abundances of *Succinivibrionaceae* ($P = 0.04$) and *Muribaculaceae* ($P = 0.002$) through competition among bacterial communities. However, we observed no difference ($P > 0.10$) for the relative abundances of *Ruminococcaceae*, *Lachnospiraceae*, *Acidaminococcaceae,* and *Christensenellaceae* among treatments. Further assessment of ruminal bacteria at the genus level showed that the most abundant bacterial genus in all ruminal samples was *Prevotella* 1, in accordance with previously published literature (Mu et al., 2019). In contrast, Guo et al. (2019) found that *Rikenellaceae* RC9 gut group was the most abundant bacterial genus in all ruminal samples when cows were fed corn stover as a major roughage. This finding indicates that the dominant bacterial genera in the rumen might be changed when cows were fed different dietary treatments. Ruminal *Prevotella* 1 ($P = 0.001$) increased linearly in relative abundance with increasing FCWM supplementation. *Prevotella* is believed to be an important contributor to polysaccharide degradation in the rumen, contributing to the production of ruminal propionate (Krause et al., 2003; AlZahal et al., 2017). Therefore, an increase in the relative abundance of *Prevotella* 1 may also be a potential reason for the increased ruminal propionate concentration we detected. Golder et al. (2014) also reported that an increase in *Prevotella* induced higher acidosis eigenvalues, whereas we observed no signs of

Table 8. Relative abundances (>0.5%) of the bacterial families and genera in the feces of Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
Family						
Ruminococcaceae	0.30	0.32	0.36	0.02	0.08	0.64
Peptostreptococcaceae	0.18	0.15	0.10	0.03	0.09	0.85
Rikenellaceae	0.12	0.13	0.12	0.02	0.91	0.71
Lachnospiraceae	0.09	0.09	0.09	0.009	0.70	0.93
Prevotellaceae	0.06	0.07	0.10	0.01	0.04	0.33
Christensenellaceae	0.05	0.05	0.05	0.005	0.79	0.62
Muribaculaceae	0.03	0.03	0.03	0.003	0.27	0.62
Bifidobacteriaceae	0.03	0.03	0.01	0.01	0.17	0.81
Akkermansiaceae	0.02	0.02	0.02	0.004	0.75	0.56
Bacteroidaceae	0.01	0.02	0.02	0.004	0.19	0.87
Clostridiaceae 1	0.01	0.01	0.01	0.003	0.35	0.46
Family XIII	0.01	0.01	0.01	0.001	0.31	0.56
Erysipelotrichaceae	0.01	0.01	0.008	0.002	0.15	0.88
Others	0.05	0.07	0.07	0.005	0.11	0.38
Genus						
Ruminococcaceae UCG-005	0.14	0.16	0.18	0.01	0.09	0.79
Rikenellaceae RC9 gut group	0.10	0.11	0.10	0.02	0.98	0.62
Romboutsia	0.09	0.07	0.05	0.02	0.11	0.87
Paeniclostridium	0.09	0.08	0.05	0.02	0.15	0.63
Prevotella 1	0.04	0.03	0.06	0.01	0.13	0.26
Christensenellaceae R-7 group	0.05	0.05	0.05	0.004	0.76	0.57
Ruminococcaceae UCG-013	0.03	0.02	0.03	0.003	0.37	0.34
Bifidobacterium	0.04	0.03	0.01	0.01	0.18	0.77
Ruminococcaceae UCG-010	0.01	0.02	0.02	0.004	0.13	0.82
Prevotellaceae UCG-003	0.02	0.02	0.02	0.004	0.75	0.33
Akkermansia	0.02	0.02	0.02	0.004	0.75	0.57
Bacteroides	0.009	0.02	0.02	0.005	0.29	0.89
Others	0.36	0.37	0.38	0.01	0.15	0.81

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.

acidosis after feeding FCWM to cows. This finding agreed with a report by Mohammed et al. (2012), who found that *Prevotella* were not related directly to ruminal acidosis. *Rikenellaceae* RC9 gut group ($P = 0.06$), *Selenomonas* 1 ($P = 0.03$) and *Candidatus Saccharimonas* ($P = 0.01$) increased linearly in relative abundance at the genus level with increasing FCWM supplementation. In contrast, the relative abundances of *Prevotella* 7 ($P = 0.04$), *Succinivibrionaceae* UCG-001 ($P = 0.03$), and *Lachnospiraceae* NK3A20 group ($P = 0.08$) decreased linearly. The other rumen bacterial genera did not differ in abundance among treatments ($P > 0.10$).

Clustering results showed a total of 13 phyla, 21 classes, 29 orders, 54 families, 175 genera, and 181 species of bacteria in the fecal samples. The results for the relative abundances (>0.5%) of the bacterial families and genera in the feces are given in Table 8. In contrast to the composition of the rumen bacterial community, the most abundant bacterial family in the fecal samples was *Ruminococcaceae*, followed by *Peptostreptococcaceae* and *Rikenellaceae*. In agreement with our results, Petri et al. (2019) found that the *Ruminococcaceae* family was the most abundant fecal bacteria of dairy cows, regardless of dietary treatment. The relative abundances of *Ruminococcaceae* ($P = 0.08$) and *Prevotellaceae* ($P = 0.04$) at the family level increased linearly with increasing FCWM supplementation. Similarly, *Ruminococcaceae* UCG-005 ($P = 0.09$) increased linearly in relative abundance at the genus level with increasing FCWM supplementation. These increased bacterial abundances might have contributed to improvements in the degradation of nutrients in the intestine (Krause et al., 2003; Petri et al., 2019). In contrast, *Peptostreptococcaceae* ($P = 0.09$) decreased linearly in relative abundance with increasing FCWM supplementation, but its significant function warrants further investigation. We observed no difference ($P > 0.10$) for the relative abundances of other fecal bacterial families and genera among treatments.

Bacterial Diversity. The results of the rarefaction curve analysis showed that the number of OTU in ruminal fluids and fecal samples from each group showed a trend of increasing first and then becoming stable with increasing sequence number (Figure 1A, B), indicating that the sample sequences were sufficient and could be used for diversity analysis. As shown in Table 9, the coverage values in ruminal fluids and fe-

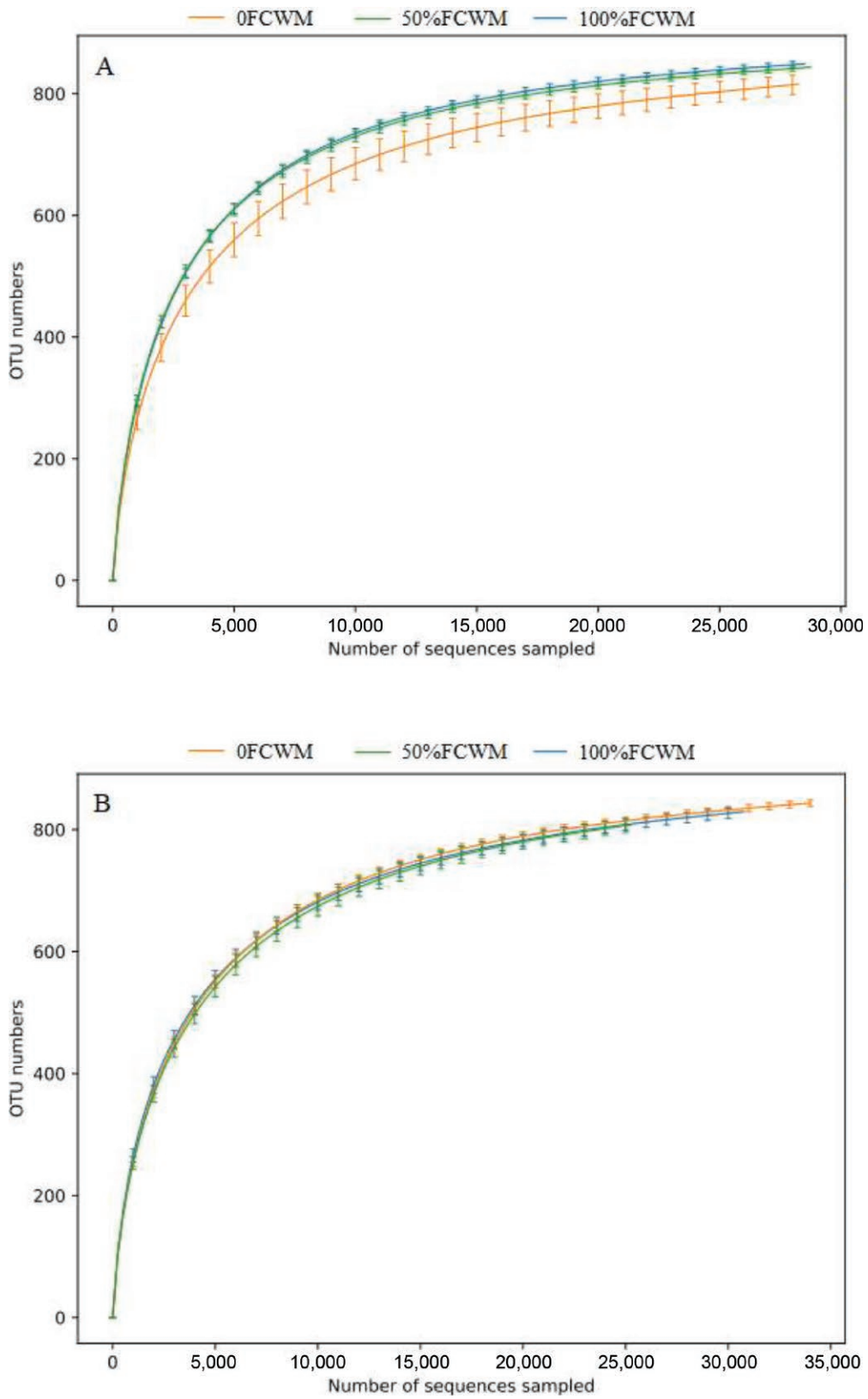

Figure 1. Rarefaction curves of the operational taxonomic units (OTU) in (A) ruminal fluid, and (B) feces of cows fed 3 diets. 0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture. Error bars represent SD.

Table 9. Microbial diversity indices for the bacterial communities in the ruminal fluids and feces of Holstein cows fed 3 diets

Item	Treatment[1]			SEM	*P*-value	
	0FCWM	50%FCWM	100%FCWM		Linear	Quadratic
Ruminal fluid						
OTU[2]	832.8	850.2	861.1	8.84	0.01	0.71
Coverage	0.99	0.99	0.99	0.0003	0.70	0.48
Shannon index	4.85	5.28	5.24	0.16	0.09	0.25
Simpson index	0.05	0.02	0.02	0.01	0.06	0.27
Ace index	864.6	867.9	877.2	4.92	0.02	0.50
Chao index	873.1	874.3	882.3	5.71	0.12	0.49
Feces						
OTU[2]	862.9	846.1	851.3	10.2	0.27	0.23
Coverage	0.99	0.99	0.99	0.0002	0.30	0.15
Shannon index	4.82	4.87	5.09	0.12	0.13	0.55
Simpson index	0.04	0.03	0.03	0.006	0.17	0.98
Ace index	874.2	872.9	877.2	4.90	0.55	0.53
Chao index	874.8	877.3	879.1	6.48	0.51	0.94

[1]0FCWM = basal diet; 50%FCWM = 50% replacement of soybean meal with fermented corn gluten-wheat bran mixture; 100%FCWM = 100% replacement of soybean meal with fermented corn gluten-wheat bran mixture.
[2]OTU = operational taxonomic unit.

cal samples from each group were approximately 0.99, indicating that the sequencing results could reflect the true situation of the bacteria in the samples. The numbers of bacterial OTU ($P = 0.01$) and Shannon ($P = 0.09$) and Ace indices ($P = 0.02$) in the ruminal fluids increased linearly with increasing FCWM supplementation, whereas the ruminal Simpson index ($P = 0.06$) decreased linearly. These results indicated that FCWM might have the potential to increase the ruminal bacterial diversity of cows. Improvement in ruminal bacterial diversity is considered ecologically beneficial and indicates a healthy microbiome, which has a beneficial effect for the host (Lozupone et al., 2012). However, the ruminal Chao index was not affected by the FCWM treatments ($P > 0.10$). Furthermore, the fecal bacterial OTU and Simpson, Ace, Shannon, and Chao indices were not affected by the FCWM treatments ($P > 0.10$), indicating that a difference in diversity was not found.

CONCLUSIONS

As hypothesized, the replacement of SBM by FCWM resulted in higher nutrient digestibility and increased propionate, as well as total VFA production, which in turn resulted in a higher energy and MCP supply for lactation and higher milk, lactose and milk protein yields in FCWM-fed cows. Furthermore, the replacement of SBM by FCWM also altered the ruminal bacterial community composition of cows, which may have been beneficial for milk production. Overall, the present study provides a data reference for the use of FCWM in dairy production; FCWM may be an available protein source for nutritionists to formulate ruminant diets.

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