Effects of a high-protein corn product compared with soy and canola protein sources on nutrient digestibility and production responses in mid-lactation dairy cows

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ABSTRACT

An experiment was conducted to assess the effects of a novel and proprietary high-protein corn product [56% crude protein (CP)] relative to other common sources of protein on the lactation performance of dairy cows. Twenty-four Holstein cows (620 ± 47.7 kg of body weight, 111 ± 34 d in milk, 2.28 ± 0.46 lactations; mean $\pm$ standard deviation) were randomly assigned to treatment sequence in a replicated 4×4 Latin square design balanced for carryover effects. Cows were individually fed 1 of 4 diets with a different protein concentrate source during each 28-d period, including soybean meal (SBM), high-protein corn product (HPCP), soybean meal with rumen-bypass soy protein (SBMBP), and canola meal with rumen-bypass soy protein (CANBP). Diets were formulated for equal concentrations of CP and balanced to meet predicted lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), whereas SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. Data were analyzed using mixed models with the fixed effects of treatment, period, square, the interactions of treatment and period and of treatment and square, and the random effect of cow. The CANBP diet increased dry matter intake (DMI) compared with SBM and HPCP. Treatment affected milk yield, as SBMBP and CANBP increased yield compared with SBM, but HPCP decreased milk yield compared with all treatments. The HPCP diet reduced CP intake as a percent of total DMI and increased the CP content oforts, indicative of selection against HPCP. The HPCP diet also decreased apparent total-tract and CP digestibility, leading to less urine nitrogen excretion and greater fecal nitrogen output. The SBMBP and CANBP diets performed similarly in nearly every variable measured, except that SBMBP increased milk urea nitrogen. In conclusion, the HPCP

diet reduced yield of milk and milk components, likely because of reduced apparent total-tract dry matter and CP digestibility.

Key words: milk yield, dietary protein, amino acids

INTRODUCTION

Corn grain is a useful feed ingredient in lactating dairy cow diets, serving as a readily available energy substrate; however, it is low in CP (approximately 7%). Through corn processing to produce value-added products, such as ethanol or corn sweeteners, the resulting co-products possess greater concentrations of protein and have utility as protein sources for livestock (Loy and Lundy, 2019).

Corn wet-milling and bioethanol production are 2 common processes that generate high-protein corn by-products (Loy and Lundy, 2019). Wet-milling removes the carbohydrate component through various mechanisms, resulting in the production of corn gluten feed (25% CP) and corn gluten meal (60% CP; Loy and Lundy, 2019). Gluten meal is a high-value product destined for feed ingredients in pet, poultry, and swine diets, whereas corn gluten feed is primarily an ingredient for ruminant diets (Loy and Lundy, 2019). Bioethanol production most commonly uses starch in grains for fermentation. The fermentation process results in spent grains known as distillers grains with solubles (DGS), which have a CP concentration of 27 to 31% (Belyea et al., 2004; Liu, 2011). Removal of fat from DGS marginally increases CP concentration (34%; Wang et al., 2007; Morris et al., 2018), and removal of bran and germ components enhances the efficiency of ethanol production and increases CP concentration to more than 40% (Corredor et al., 2006; Robinson et al., 2008). Distillers grains have successfully been fed to beef (Klopfenstein et al., 2008) and dairy cattle (Kelzer et al., 2009; Schingoethe et al., 2009) as a protein source, and the inclusion has become more common as ethanol production grows.

More nascent technologies are being evaluated to further add value to DGS. One concept uses sieving

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and elutriation to separate particles based upon size and density, increasing protein concentration up to 40% (Srinivasan et al., 2005). Distillers grains are an attractive feedstock for second-generation, fiber-based ethanol production because of its fiber content and ability to streamline production processes (White et al., 2008; Kim et al., 2010; Mikulski and Kłosowski, 2018), producing a co-product of 34 to 50% CP (Kim et al., 2008; Lundy et al., 2015). Ability to cost-effectively integrate various technologies into existing production processes will ultimately determine viability of next-generation ethanol plants in the future, and the feed value of resulting co-products is critical.

A new, proprietary high-protein corn product with 56% CP may be a novel option for lactating dairy cow diets. With very limited data available on livestock feeding performance using this new technology, we chose to determine its utility for replacing commonly fed protein sources in North America, such as soybean meal and canola meal, as has been done with other high-protein corn products (Nichols et al., 1998; Mulrooney et al., 2009; Christen et al., 2010). Care must be taken when feeding different protein sources, because not all protein sources have equal value, due to differences in their AA profiles relative to the AA requirements of the animal. The 2 most limiting AA in the lactating dairy cow are typically considered to be lysine and methionine (NRC, 2001). Lysine concentration as a percentage of CP is greatest in soybean meal and least in corn products, whereas methionine concentration is greatest in canola meal and least in soybean meal (Maxin et al., 2013). When diets are formulated for similar CP concentration, the lack of methionine or lysine may inhibit performance, depending on diet characteristics (Donkin et al., 1989; Nichols et al., 1998; Wang et al., 2010). Therefore, the objective of this experiment was to assess milk production and nitrogen digestibility responses of high-producing Holstein cows following replacement of common protein sources with a novel high-protein corn product, while maintaining consistent predicted supply of metabolizable methionine and lysine across diets. We hypothesized that the novel high-protein corn product would perform similarly to other common protein sources fed in dairy diets.

MATERIALS AND METHODS

Animals, Design, and Diets

Twenty-four multiparous Holstein cows near peak lactation (111 ± 34 DIM, 2.3 ± 0.46 lactations, mean $\pm$ SD) from the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) were randomly assigned to treatment sequence using a random number

sequence generator (Microsoft Excel, Microsoft Corporation, Redmond, WA) in a replicated 4×4 Latin square design balanced for carryover effects. Power analysis conducted before study initiation determined that 24 animals were sufficient to detect a difference in mean milk yield of 2.7 kg/d ($\alpha = 0.05$, $\beta = 0.16$; Kononoff and Hanford, 2006). Cows were housed in a tie-stall barn. All experimental procedures were conducted with approval from the Institutional Animal Care and Use Committee at Kansas State University. Treatment periods were 28 d, with 25 d for diet adaptation and 3 d for sample collection. Feeding of treatments began in October 2018 and continued through January 2019. Cows were fed twice daily at 0600 and 1900 h and milked 3 times daily at 0400, 1100, and 1800 h.

Cows were individually fed 1 of 4 diets ad libitum, with different protein concentrate sources, during each 28-d period, including soybean meal (**SBM**), high-protein corn product (**HPCP**; 56.1% CP), soybean meal with bypass protein (**SBMBP**), and canola meal with bypass protein (**CANBP**). Soybean meal and canola meal were chosen as treatments for comparison because they are typical protein sources fed on North American farms and can be used as standard benchmarks for comparison with the novel HPCP. A base TMR was delivered to each cow, with treatment top-dressed and mixed by hand at each feeding. Diets were formulated (NDS Professional, RUM&N Sas, Reggio Emilia, Italy) to meet nutrient requirements of a 629-kg cow producing 52 kg/d milk with 3.6% fat and 3.1% protein. Diets were formulated for equal concentrations of CP and balanced to meet metabolizable lysine (79.5 g/d) and methionine (225 g/d) requirements. Amino acid composition of the HPCP (Official Method 982.30; AOAC International, 2016) is shown in Table 1, whereas library values were used for all other common feed ingredients (NRC, 2001). The SBM diet was formulated to provide 5.7% RUP, and SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. The HPCP RUP was estimated in commercial laboratories with a combination of 16-h incubation in situ (Calamiglia and Stern, 1995) and in vitro (Ross et al., 2013), and intestinal digestibility was determined with the modifications of Ross et al. (2013). The diets were formulated to provide equal concentrations of forage NDF and starch. Nutrient composition and ingredients are shown in Table 2.

One cow was removed from the experiment during the second collection period due to hemorrhagic diarrhea and was subsequently replaced for the remaining 2 periods. Period 2 data were not retained from this cow. Another cow was dried off in 1 quarter following an udder injury during period 3. Data were analyzed both with and without inclusion of this cow post-dry off, and

Table 1. Amino acid profile of a proprietary high-protein corn product (HPCP) fed to lactating Holstein cows

Item	HPCP
CP, %	56.1
AA, g/kg of DM	
Ala	31.7
Arg	12.9
Asp	27.6
Glu	67.5
His	9.1
Ile	17.7
Lys	8.7
Met	8.4
Phe	20.9
Pro	33.2
Ser	18.2
Thr	16.0
Tyr	18.3
Val	21.9

it was determined that this did not affect evidence of significance for any treatment, so data were included in the final analysis. This resulted in analysis of data from

95 of 96 total collection period observations, with only 23 of 24 observations for HPCP, due to the removal of 1 cow during the collection period.

Data and Sample Collection and Analysis

Feed offered and refused for each cow was recorded daily during the final 3 d of each treatment period. Water intake was recorded daily. Samples of the TMR, concentrate treatment mixes, and individual feed ingredients were collected daily and composited over the collection period for analysis. Feed refusals were frozen and later composited over the collection period for each cow and dried in a 55°C forced-air oven. All feed samples were sent to Dairyland Laboratories Inc. (Arcadia, WI) for nutrient analysis (Table 3) using wet chemistry. Dry matter was determined by drying at 60°C until moisture was less than 6%, and then at 105°C for 3 h (Shreve et al., 2006). Neutral detergent fiber was determined using an amylase-treated method (method 2002.04; AOAC International, 2016). Indigestible NDF

Table 2. Ingredients and nutrient composition of experimental diets fed to lactating Holstein cows supplemented with a protein source from either soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

Item, % of DM	Diet			
	SBM	HPCP	SBMBP	CANBP
Dietary ingredients				
Corn silage	34.5	34.5	34.5	34.5
Alfalfa hay	19.5	19.5	19.5	19.5
Soybean meal, 47.5% solvent extracted	10.7	—	3.9	—
High-protein corn product ¹	—	9.4	—	—
Canola meal, 37% solvent extracted	—	—	—	8.9
Expeller soybean meal ²	—	—	8.0	4.7
Ground corn grain	22.6	22.6	22.6	22.6
Soybean hulls	7.8	8.8	6.8	5.0
Ca salts of long-chain fatty acids ³	0.75	0.75	0.75	0.75
Lysine hydrochloride ⁴	0.32	0.68	0.11	0.26
HMBi ⁵	0.24	0.17	0.17	0.15
Micronutrient premix ⁶	3.6	3.7	3.6	3.6
Diet nutrient composition				
DM, % as fed	52.8	52.6	52.9	52.9
CP	16.8	16.8	16.8	16.8
aNDFom ⁷	30.2	30.3	30.0	30.3
Starch	22.5	22.7	22.6	22.7
Ether extract	4.3	4.9	4.5	4.7
Ash	8.2	8.1	8.2	8.3

¹Proprietary high-protein corn product.

²SoyPlus (Landus Cooperative, Ames, IA).

³Megalac-R (Arm and Hammer Animal Nutrition, Princeton, NJ).

⁴Lysine hydrochloride, lecithin, and hydrogenated vegetable oil, AjiPro-L (Ajinomoto Animal Nutrition North America, Chicago, IL).

⁵Isopropyl ester of 2-hydroxy-4-methylthio butanoic acid, MetaSmart (Adisseo Inc., Antony, France).

⁶The premix consists of 30.5% limestone, 20.8% sodium bicarbonate, 30.5% Kruse lactation premix (International Nutrition, Omaha, NE), 4.40% trace mineral salt, 4.42% white salt, 7.08% magnesium oxide, 4.42% vitamin E premix, 0.69% Zinpro 4-plex (Zinpro Corporation, Eden Prairie, MN), 0.35% Zinpro 120 (Zinpro Corporation), and 0.19% Rumensin 90 (Elanco Animal Health, Greenfield, IN).

⁷aNDFom = ash-free, amylase-treated NDF.

Table 3. Nutrient composition of major feed ingredients fed to lactating Holstein cows

Component, % of DM	Feed ingredient ¹						
	Corn silage	Alfalfa hay	Grain mix	SBM ²	HPCP ²	SBMBP ²	CANBP ²
DM, % as fed	29.1	92.7	90.6	91.7	87.5	92.4	92.1
CP	9.3	23.6	9.3	36.1	35.8	35.6	35.7
aNDFom ³	47.0	33.5	16.3	16.5	17.1	15.7	17.1
Starch	16.6	2.3	49.5	12.8	13.6	13.5	13.9
Ether extract ⁴	4.2	2.2	3.9	7.7	10.9	8.9	9.7
Ash	5.9	10.1	10.5	6.8	6.1	6.5	7.4

¹SBM = soybean meal top-dress; HPCP = high-protein corn product top-dress; SBMBP = soybean meal + bypass protein top-dress; CANBP = canola meal + bypass protein top-dress.

²Protein top-dress treatments included 16.4% dry ground corn, 4.3% calcium salts of long-chain fatty acids, soy hulls, AjiPro-L (Ajinomoto Animal Nutrition North America Inc., Chicago, IL), and MetaSmart (Adisseo Inc., Antony, France).

³aNDFom = ash-free, amylase-treated NDF.

⁴Protein top-dress treatments were subjected to an additional acid hydrolysis step due to inclusion of calcium salts of fatty acids.

was determined in fermentation flasks according to Goering and Van Soest (1970) after 240-h fermentation (Raffrenato et al., 2018), and residual NDF was determined as described above. Crude protein was determined via analysis for nitrogen with AOAC method 990.03 (AOAC International, 2016), and acid-detergent insoluble crude protein (**ADICP**) was determined on grain treatments by collecting ADF residue on filter paper using method 973.18 (AOAC International, 2016) and analyzing for nitrogen to determine CP. Crude fat was determined with the use of diethyl ether on a Foss Soxtec 2047 (Foss North America, Eden Prairie, MN; method 920.39, AOAC International, 2016), and grain treatments containing calcium salts of fatty acids were subjected to an additional acid hydrolysis step using the SoxCap 2047 (Foss North America). Ash concentration was determined using AOAC method 942.05 (AOAC International, 2016). Orts samples were analyzed for 240-h indigestible NDF and CP using the same methods.

Milk samples were collected at every milking during the collection period. Samples were analyzed for fat, true protein, lactose, nonfat solids, and MUN using infrared spectroscopy (Bentley FTS; Bentley Instruments Inc., Chaska, MN), and somatic cells were counted using a flow cytometer (Bentley FCM; Bentley Instruments Inc.) at MQT Labs (Kansas City, MO). The 3.5% FCM was calculated as $\text{FCM} = (\text{milk kg} \times 0.432) + (\text{fat kg} \times 16.216)$, and ECM = $(0.327 \times \text{milk kg}) + (12.95 \times \text{fat kg}) + (7.65 \times \text{protein kg})$.

Fecal grab samples were collected for determination of total-tract digestibility every 9 h during the 3-d collection period to represent every 3 h over a 24-h period. Samples were frozen immediately and were later thawed and composited by cow on an equal volume basis and dried in a 55°C forced-air oven. Dried samples were later ground through a 6-mm screen for analysis by Dairyland Laboratories. Indigestible NDF was deter-

mined in fermentation flasks according to Goering and Van Soest (1970) after 240-h fermentation (Raffrenato et al., 2018), and residual NDF was determined as outlined above. Crude protein was determined by analysis for nitrogen using AOAC method 990.03. Total-tract digestibility was calculated using 240-h indigestible NDFom as an internal marker to estimate total fecal output, with digestibility determined by the ratio of nutrient concentration in the feces and diet consumed (Cochran and Galyean, 1994).

Urine samples were collected via vulva stimulation every 9 h during the 3-d collection period to represent every 3 h over a 24-h period. Urine was diluted 1:3 in 0.15 N H₂SO₄ to achieve pH <2 and then frozen at -20°C. After each collection period, samples were thawed and composited by cow and period on an equal volume basis. Urine was analyzed in duplicate for creatinine using alkaline picrate (Cayman Chemical, Ann Arbor, MI), and total urine output was estimated assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urine N concentration was determined in duplicate using a LECO TruMac Nitrogen Analyzer (LECO Corporation, St. Joseph, MO). Total daily urinary N excretion was calculated using the estimated volume of urine excretion from creatinine analysis.

Cows were weighed individually on the initial 2 d of the study and during the final 2 d of each collection period to determine body weight change. Body condition score was recorded for each cow by 2 trained investigators blinded to treatment at the initiation of the study and at the end of each period to determine body condition change.

Statistical Analysis

During period 1, some individual milk weight data were missing due to an equipment recording error. To

account for missing data in an unbiased manner, milk yield, component yield, and composition data were analyzed by individual milking over the 3-d collection period. Data were analyzed using JMP (version 13.0, SAS Institute Inc., Cary, NC) according to the following statistical models. The model for milk production data was

$$Y_{ijkln} = \mu + T_i + P_j + S_k + T_i \times P_j + T_i \times S_k \\ + M_l(P_j) + C_n + C_n \times P_j + e_{ijkln},$$

where μ is the overall treatment mean, T_i is the fixed effect of treatment ($i = 1$ to 4), P_j is the fixed effect of period ($j = 1$ to 4), S_k is the fixed effect of square ($k = 1$ to 6), $T_i \times P_j$ is the interaction of treatment and period, $T_i \times S_k$ is the interaction of treatment and square, $M_l(P_j)$ is the random effect of milking time nested within period ($l = 1$ to 9), C_n is the random effect of cow ($n = 1$ to 24), $C_n \times P_j$ is the interaction of cow and period, and e_{ijkln} is the residual error.

The model for feed and water intake, feed efficiency, nitrogen balance and output, digestibility, body weight, and body condition score data was as follows:

$$Y_{ijkn} = \mu + T_i + P_j + S_k + T_i \times P_j + T_i \\ \times S_k + C_n + e_{ijkn},$$

where μ is the overall treatment mean, T_i is the fixed effect of treatment ($i = 1$ to 4), P_j is the fixed effect of period ($j = 1$ to 4), S_k is the fixed effect of square ($k = 1$ to 6), $T_i \times P_j$ is the interaction of treatment and period, $T_i \times S_k$ is the interaction of treatment and square, C_n is the random effect of cow ($n = 1$ to 24), and e_{ijkn} is the residual error.

Best linear unbiased predictors for cow $\times$ period milk yields were used to determine daily means for FCM and milk nitrogen excretion to calculate feed efficiency and nitrogen balance. Denominator degrees of freedom were checked to verify the lack of pseudoreplication in all models.

Data were evaluated for normality, and data points with studentized residuals >4 or <-4 were considered outliers and removed from analysis. Interactions for fixed effects with $P > 0.20$ were removed from the model. Differences between treatments were declared significant when $P < 0.05$ according to Tukey's range test.

RESULTS

DMI

Treatment affected DMI, with CANBP increasing DMI compared with SBM and HPCP (Table 4; $P < 0.01$), and HPCP increased CP concentration compared with all other treatments (Table 4; $P < 0.001$). These 2 factors altered N intake, as both SBMBP and CANBP increased N intake compared with HPCP (Table 4; $P < 0.001$). HPCP reduced CP intake as a proportion of total DMI compared with all other treatments (Table 4; $P < 0.001$). The HPCP and SBMBP diets increased water consumption relative to CANBP (Table 4; $P < 0.001$).

Milk Yield and Components

Treatment affected milk yield (Table 5; $P < 0.001$), as cows fed SBM produced less milk than those fed SBMBP and CANBP but more than those fed HPCP. We found no evidence of differences in milk fat concentration (Table 5; $P = 0.86$), but diet affected milk fat yield (Table 5; $P < 0.001$). We found that CANBP increased milk fat yield compared with SBM and HPCP, and SBMBP increased fat yield compared with HPCP. The SBM diet increased milk protein concentration compared with HPCP (Table 5; $P < 0.01$), and HPCP decreased protein yield compared with all other treatments (Table 5; $P < 0.01$). Analysis revealed no evidence of treatment effects on milk lactose concentration (Table 5; $P = 0.59$), but HPCP decreased lactose yield ($P < 0.001$). The SBM and SBMBP diets increased

Table 4. Dry matter and water intake of lactating Holstein cows supplemented with a protein source consisting of soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

Item	Diet				SEM	P-value
	SBM	HPCP	SBMBP	CANBP		
DMI, kg/d	27.6 ^b	27.3 ^b	28.1 ^{ab}	28.9 ^a	0.46	<0.01
Orts CP, %	15.5 ^b	18.8 ^a	14.6 ^b	15.0 ^b	0.37	<0.001
N intake, g/d	753 ^{ab}	722 ^b	762 ^a	783 ^a	12.7	<0.001
CP intake, % of total DMI	17.0 ^a	16.5 ^b	17.0 ^a	16.9 ^a	0.04	<0.001
Water intake, L/d	108.5 ^{ab}	110.4 ^a	111.9 ^a	102.8 ^b	3.0	<0.001

^{a,b}Means with different superscripts within a row are significantly different by Tukey's honestly significant difference ($P < 0.05$).

Table 5. Production of milk and milk components and milk nitrogen secretion of lactating Holstein cows supplemented with a protein source consisting of soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

Item	Diet				SEM	P-value
	SBM	HPCP	SBMBP	CANBP		
Milk, kg/d	39.9 ^b	37.4 ^c	42.0 ^a	42.4 ^a	1.79	<0.001
Fat, %	3.86	3.91	3.88	3.87	0.10	0.86
Protein, %	3.08 ^a	3.00 ^b	3.03 ^{ab}	3.04 ^{ab}	0.04	<0.01
Lactose, %	4.81	4.84	4.84	4.84	0.03	0.59
MUN, mg/dL	11.7 ^a	10.0 ^c	11.6 ^a	10.8 ^b	0.34	<0.01
SCC, ¹ 1,000 cells/mL	41.7	39.8	33.1	38.0	1.23	0.28
Fat, kg/d	1.50 ^{bc}	1.44 ^c	1.58 ^{ab}	1.61 ^a	0.05	<0.001
Protein, kg/d	1.21 ^a	1.12 ^b	1.27 ^a	1.28 ^a	0.05	<0.001
Lactose, kg/d	1.92 ^b	1.80 ^c	2.04 ^a	2.06 ^a	0.08	<0.001
SNF, kg/d	3.41 ^b	3.17 ^c	3.58 ^{ab}	3.62 ^a	0.15	<0.001
FCM, kg/d	41.5 ^b	39.4 ^b	43.8 ^a	44.4 ^a	1.50	<0.001
ECM, kg/d	41.7 ^b	39.3 ^c	43.9 ^{ab}	44.4 ^a	1.54	<0.001
Feed efficiency, kg of FCM/kg of DMI	1.52 ^{ab}	1.49 ^b	1.56 ^a	1.52 ^{ab}	0.03	0.06
Milk nitrogen secretion, g/d	198 ^a	180 ^b	204 ^a	207 ^a	8.2	<0.001

^{a-c}Means with different superscripts within a row are significantly different by Tukey's honestly significant difference ($P < 0.05$).

¹Data were log₁₀-transformed for analysis, and means were reverse transformed.

MUN compared with CANBP (Table 5; $P < 0.01$), but HPCP decreased MUN. We discovered no evidence of a treatment effect on SCC (Table 5; $P = 0.41$). Feeding HPCP reduced milk nitrogen secretion compared with all other treatments (Table 5; $P < 0.001$).

Cows fed SBMBP and CANBP produced more FCM compared with those fed SBM and HPCP (Table 5; $P < 0.001$). Treatment also affected ECM (Table 5; $P < 0.001$): CANBP increased ECM yield compared with SBM and HPCP. Cows fed HPCP produced the least ECM. A tendency for a treatment effect on feed efficiency occurred (FCM:DMI; Table 5; $P = 0.06$);

SBMBP increased feed efficiency compared with HPCP ($P < 0.05$, Tukey's HSD).

Digestibility and Nitrogen Balance

The CANBP diet increased urinary N concentration compared with HPCP (Table 6; $P < 0.01$). We found no evidence of a treatment effect on urine output (Table 6; $P = 0.15$); however, HPCP reduced urinary N excretion compared with SBM and CANBP (Table 6, $P = 0.02$).

Compared with SBMBP and CANBP, SBM reduced fecal N concentration and HPCP increased fecal N con-

Table 6. Urine and feces nitrogen excretion, whole-body nitrogen balance, apparent total-tract DM and CP digestibility, and BW and BCS change of lactating Holstein cows supplemented with protein source consisting of soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

Item	Diet				SEM	P-value
	SBM	HPCP	SBMBP	CANBP		
Urine N, %	0.70 ^{ab}	0.63 ^b	0.69 ^{ab}	0.76 ^a	0.03	<0.01
Urine output, ¹ L/d	49.4	40.9	45.0	44.9	2.56	0.15
Urine N excretion, g/d	348 ^a	255 ^b	308 ^{ab}	338 ^a	23.9	0.02
Fecal N, % DM	2.7 ^c	3.1 ^a	2.8 ^b	2.8 ^b	0.02	<0.001
Fecal output, ² kg DM/d	9.8 ^a	10.2 ^a	9.8 ^a	10.4 ^a	0.22	0.04
Fecal N excretion, g/d	264 ^c	313 ^a	270 ^{bc}	287 ^b	6.8	<0.001
N balance, ³ g/d	-56	-29	-22	-53	25.1	0.65
Apparent total-tract DM digestibility, ⁴ %	64.7 ^a	62.8 ^b	65.2 ^a	64.2 ^a	0.34	<0.001
Apparent total-tract N digestibility, ⁵ %	65.1 ^a	56.5 ^c	64.6 ^{ab}	63.4 ^b	0.47	<0.001
Body weight change, kg/28 d	17.9	14.4	17.0	17.2	3.11	0.89
BCS change, units/28 d	0.03	0.01	0.07	0.11	0.04	0.19

^{a-c}Means with different superscripts within a row are significantly different by Tukey's honestly significant difference ($P < 0.05$).

¹Estimated assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

²Estimated using 240-h indigestible NDF as an internal marker (Cochran and Galyean, 1994).

³Nitrogen balance = N intake - (milk N + urine N + fecal N).

⁴Apparent total-tract digestibility = $100 - 100 \times (\text{g of fecal DM} \div \text{g of DM consumed})$; Cochran and Galyean (1994).

⁵Apparent total-tract N digestibility = $100 - 100 \times (\text{g of fecal N} \div \text{g of N consumed})$; Cochran and Galyean (1994).

centration (Table 6; $P < 0.001$). Results of an F -test for fecal DM output were significant ($P = 0.04$); however, the conservative Tukey's HSD did not identify any significant contrast among treatments. Cows fed HPCP excreted more N than all other treatments (Table 6; $P < 0.001$), and SBM decreased fecal N excretion compared with CANBP.

The HPCP diet reduced total-tract DM digestibility compared with all other treatments (Table 6; $P < 0.001$). Treatment also affected apparent total-tract N digestibility, as CANBP reduced digestibility compared with SBM but increased digestibility compared with HPCP (Table 6; $P < 0.001$). We discovered no evidence of a treatment effect on N balance (Table 6; $P = 0.65$), body weight change ($P = 0.89$), nor BCS change ($P = 0.19$).

Treatment Nutrient Profile

Post-hoc analysis of top-dress premixes was carried out to confirm that formulated nutrient profiles were achieved and to evaluate whether Maillard product formation may have contributed to HPCP responses. The results revealed a dramatically greater concentration of ADICP in the HPCP mix (7.9%) compared with all other treatments (1.0% or less; data not shown).

DISCUSSION

The reduction of milk production among cows fed the HPCP diet appears to result from 2 factors: first, through the reduction of CP intake, and second, through the reduction in apparent total-tract CP digestibility. The cows fed HPCP had greaterorts CP concentration and lower CP intake as a percentage of total DMI compared with the other treatments, suggesting that they sorted against the HPCP. Although most mature dairy cattle sort during lactation to obtain a more energy-dense diet (Miller-Cushon and DeVries, 2017), instances arise where cattle sort against protein concentrate sources due to palatability (Lardy and Kerley, 1994). It may be that the production of HPCP imparted a flavor or odor prompting the cows to select against the HPCP, as corn coproducts are typically highly palatable (Klopfenstein et al., 2008).

The bypass protein was added to the SBMBP and CANBP diets in this study to assess the HPCP on an equal-RUP basis, which was not possible with the SBM diet alone. In the present study, the HPCP diet decreased total-tract apparent N digestibility and urinary N excretion, and increased fecal N excretion, compared with the other treatments. In other studies, increasing RUP decreased apparent CP digestibility in mid-lactation cows, and cows fed diets with greater

RUP had reduced urinary N excretion and elevated fecal N excretion despite similar N intake (Brito and Broderick, 2007). This suggests that the ruminal digestibility of the HPCP in our study was perhaps less than anticipated, leading to the greater fecal N excretion and lesser urinary N excretion. The results from Brito and Broderick (2007) suggest that the lesser fecal N excretion of the SBM diet compared with all other treatments was likely due to the lesser RUP content of that diet. Additionally, Noftsker and St-Pierre (2003) reported a decrease in milk production from cows fed less-digestible RUP sources at the same dietary CP concentration, which aligns with the lesser milk production of HPCP compared with the other treatments in this study.

One explanation for the reduction in digestibility of CP that led to reduced milk production for HPCP compared with other treatments may be the formation of Maillard products during drying. Excessive heating of feed products is known to increase ADICP concentration in distillers grains, which is positively correlated with fecal N concentration (van Soest and Mason, 1991). Previous HPCP protein digestibility analysis provided by the suppliers using *in situ* (Ross et al., 2013) and *in vitro* methods (Krishnamoorthy et al., 1983) conducted in commercial laboratories reported 52.9% RUP. Content of ADICP was unknown. Nonetheless, post-hoc analysis of the protein source top-dresses in the present study clearly demonstrated elevated ADICP for the HPCP top-dress. The HPCP top-dress was 7.9% ADICP, whereas the other top-dress protein treatments were less than 1.0% ADICP (data not shown). This coincides with the high fecal N excretion for those cows and the dark color of the product (Figure 1), consistent with Maillard product formation (Urriola et al., 2013). Similarly, bench-top production of a high-protein corn product during the initial concept development of cellulosic ethanol fermentation using distillers grains resulted in heat damage and decreased lysine and methionine concentration compared with protein from distillers grains (Kim et al., 2008). Traditional heating and cooking of the grain before the dry-milling process for ethanol production resulted in RUP concentrations (55%) similar to those of a high-protein DGS from fractionation of corn before the fermentation process, but the high-protein DGS had greater total-tract digestibility (Kelzer et al., 2010). In contrast, a high-protein corn product (39.1%) from cellulosic fermentation of distillers grains decreased DM and OM digestibility compared with traditional distillers grains (34.1% CP; Lundy et al., 2015). It is unclear whether the reduction in protein availability for HPCP is due to the drying process for long-term storage or to another upstream process, so further investigation of those processes is

Figure 1. Proprietary high-protein corn product fed to lactating Holstein cows as the sole protein concentrate source in the present study, demonstrating a dark color likely indicative of Maillard product formation.

warranted before determining whether the HPCP in this study is a feasible protein supplement for lactating dairy cows.

Milk protein concentration in this study was greater for cows fed SBM compared with HPCP. Milk protein concentration of milk is not driven by dietary CP concentration (NRC, 2001; Colmenero and Broderick, 2006a), but rather milk protein content increases as metabolizable EAA supply increases, at least until requirements are met (NRC, 2001). In the present study, dietary CP concentration was the same across diets, and all were formulated to meet lysine and methionine requirements using synthetic rumen-protected AA. The reduction in milk protein content of cows fed HPCP compared with SBM points to decreased protein quality in HPCP, especially considering the lower RUP concentration for SBM.

The supply of metabolizable protein to the cow is important for maximizing milk production, and the SBMBP and CANBP diets increased milk yield and fat-corrected milk yield compared with SBM. Similar results have been noted by others who have increased RUP content in the diet (Zanton et al., 2013), although the work of Zanton et al. (2013) did not influence DMI despite the increase in milk yield, in contrast with our study. Additionally, Broderick et al. (2009) fed greater RUP concentration, which increased fat-corrected milk through increased milk fat concentration, despite no difference in milk yield or DMI. Maintaining the correct balance of RDP and RUP is important to maximize microbial protein yield to achieve optimum milk production (Santos et al., 1998), which may explain a lack of effect of increasing RUP at constant CP concentration on milk yield in other instances (Colmenero and

Broderick, 2006b). The SBM diet was formulated to meet the Met and Lys requirements of the cow, but total MP supply was still only 95% of the requirement (data not shown), indicating that there may have been a lack of metabolizable protein to achieve the milk production of the SBMBP and CANBP diets, where the cow's predicted MP requirements were met.

Many studies have been conducted evaluating canola meal supplementation compared with soybean meal. Results have largely indicated similar performance (Sánchez and Claypool, 1983; Oba et al., 2010; Paula et al., 2018) or an increase in DMI and milk production among cows fed canola meal (Broderick et al., 2015; Gidlund et al., 2015). In this study, SBMBP and CANBP resulted in similar DMI, milk production, FCM and milk component yields, and nitrogen excretion. The only difference occurred in MUN concentration, which was greater for SBMBP than CANBP, corresponding with the data of Maxin et al. (2013), Martineau et al. (2014), and Paula et al. (2018). The meta-analysis by Martineau et al. (2014) noted that concentrations of blood AA, including essential and branched-chain AA, increase with canola meal supplementation. Although we did not measure blood AA concentrations in this study, other work has demonstrated that infusing a combination of methionine, lysine, and branched-chain AA decreased MUN without altering N efficiency (Apuhamy et al., 2011), suggesting that the canola meal in our study may have been utilized more extensively for protein synthesis. This work, combined with previous evidence mentioned earlier, reinforces the importance and interchangeability of these protein sources in dairy diets as market conditions fluctuate.

Increasing diet complexity by incorporating feeds from different plant sources may provide a more complete AA profile for the animal to enhance production. Weiss (2019) fed a diet based only on corn or a diet with other forage and protein concentrate types, and noted reduced milk production despite methionine and lysine supplementation and a similar RUP concentration, suggesting that AA other than Met and Lys may have been limiting. In contrast, adding additional protein supplement types to the diet, compared with a corn-based diet including distillers grains as a protein source, did not enhance performance in a different study (Liu et al., 2000). In the present study, the SBMBP and CANBP had more variety of ingredients than the mostly corn-based HPCP. An additional limiting AA from a single feed source may also have contributed to the reduced milk yield; however, the poor digestibility of the HPCP compared with the other treatments likely remains the primary cause.

The production process for the HPCP used in this study is proprietary; nonetheless, it is worthwhile to

Table 7. Composition of high-protein corn products reported in the literature for comparison to the current novel and proprietary product (HPCP) used in this experiment

Source	Nutrient analysis, % of DM					
	CP	ADICP ¹	NDF	ADF	Starch	EE ²
HPCP (this study)	56.1	—	4.0	3.3	2.6	1.6
Corn gluten meal						
Wohlt et al., 1991	66.6	—	—	—	—	0.7
Wohlt et al., 1991	51.8	—	—	—	—	1.8
Holter et al., 1992	66.8	—	14.5	8.3	—	1.2
High-protein distillers grains						
Hubbard et al., 2009	46.1	—	26.4	15.6	—	4.6
Kelzer et al., 2009	46.1	4.1	26.4	15.6	9.1	4.6
Reduced-fat distillers grains						
Morris et al., 2018	34.4	—	27.5	12.3	—	7.4
Ramirez-Ramirez et al., 2016	31.5	—	37.7	11.0	6.7	6.6
Secondary fermentation of distillers grains						
Lundy et al., 2015	39.1	—	32.7	15.2	1.6	7.3

¹ADICP = acid-detergent insoluble crude protein.²EE = ether extract.

compare performance of this product with that of other high-protein corn products. In contrast to the present study, feeding a high-protein, corn-based dried distillers grains with solubles, resulting from fractionation before fermentation, that was approximately 44% CP and 3.4% fat, failed to elicit a response in DMI, milk yield, ECM yield (Kelzer et al., 2009; Christen et al., 2010), or FCM yield (Kelzer et al., 2009). In the case of Hubbard et al. (2009), a high-protein corn product increased milk and FCM yields, and a high-protein distillers grain corn product decreased milk protein concentration in another case (Kelzer et al., 2009). Reduced-fat distillers grains maintained (Castillo-Lopez et al., 2014; Morris et al., 2018) or increased milk yield (Ramirez-Ramirez et al., 2016) but decreased 3.5% FCM, fat, and protein yields in a more recent study (Morris et al., 2018). Cows fed a diet with corn gluten meal as the primary protein source, compared with soybean meal, produced similar quantities of milk and FCM but produced less milk protein in early lactation (Wohlt et al., 1991). Beef cattle fed a high-protein corn product (39.1% CP) from secondary cellulosic ethanol fermentation of DGS had similar outcomes relative to a traditional dried distillers grains with solubles (34.1% CP) for final body weight and hot carcass weight but decreased back fat thickness (Lundy et al., 2015). Chemical compositions of various high-protein corn products fed to livestock are summarized in Table 7 for comparison purposes.

Investigations into the ruminal degradation parameters of high-protein corn products have demonstrated variable results. A high-protein distillers grain product from corn fractionation increased the RUP content of the supplement compared with both soybean meal and canola meal (Maxin et al., 2013), but no evidence of differences in total-tract digestibility of DM, NDF,

or CP was found in the study of Kelzer et al. (2009), in contrast with the present study. When evaluating ruminal fermentation profiles, the only effect of a high-protein corn product was a decrease of ruminal acetate concentration compared with soybean meal (Kelzer et al., 2009) or a decrease in isobutyrate concentration (Christen et al., 2010). Ruminal ammonia concentration was not affected in either instance, suggesting similar CP degradability (Kelzer et al., 2009; Christen et al., 2010). Corn gluten meal has lesser ruminal degradability compared with soybean meal (Koehn and Paterson, 1986; Annestad et al., 1987). Although ruminal fermentation profiles were not obtained in this study, the total-tract digestibility of the HPCP was clearly decreased, as indicated by the increase in fecal N excretion and decrease in urine N excretion, and ruminal degradability was most likely decreased as well.

CONCLUSIONS

Cows fed the HPCP diet produced less milk and milk components. Cows fed HPCP consumed less CP and had lower apparent total-tract and CP digestibility, suggesting that the production process may have resulted in Maillard product formation, measured as ADICP. The SBMBP and CANBP diets performed similarly when balanced for methionine and lysine supply, supporting previous data demonstrating canola meal as a valuable substitute for soybean meal. Further efforts to improve protein quality from this HPCP are warranted.

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