



Dietary replacement of soybean meal with heat-treated soybean meal or high-protein corn distillers grains on nutrient digestibility and milk composition in mid-lactation cows

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ABSTRACT

Lactation diets dependent on rumen undegradable protein (RUP) sources derived from soybean meal (SBM) products are generally high in Lys and poor in Met. We conducted an experiment to evaluate the effects of increasing dietary RUP and altering digestible AA supply by inclusion of heat-treated soybean meal (HTSBM) or high-protein corn dried distillers grains with soluble (DDGS) on performance in mid-lactation dairy cows. Twenty-four Holstein cows (200 ± 40 d in milk and 30.0 ± 3.92 kg/d of milk yield) blocked according to parity, milk yield, and days in milk were used in a 3 × 3 Latin square design experiment with 21-d periods. Treatments were (1) control (CON), a diet with 6.0% RUP containing 15.9% SBM as the main protein source; (2) HTSBM, a diet with 6.7% RUP containing 4.4% HTSBM partially replacing SBM; and (3) high-protein DDGS (FP; FlexyPro, SJC Bioenergia), a diet with 6.9% RUP containing 5.34% FP partially replacing SBM and ground corn. Diets had similar crude protein (16.9%) and net energy of lactation. Data were submitted to ANOVA using the mixed procedure of SAS software (SAS Institute Inc.). Treatment differences were evaluated using orthogonal contrasts: (1) increasing RUP (SBM vs. HTSBM + FP) and (2) altering digestible AA supply (HTSBM vs. FP). Cows fed HTSBM and FP had greater intake (values in parentheses represent treatment means of CON, HTSBM, and FP, respectively) of neutral detergent fiber (7.14, 7.35, and 7.69 kg/d), crude protein (4.27, 4.37, and 4.51 kg/d), and ether extract (0.942, 0.968, and 1.04 kg/d) compared with cows fed CON. Feeding FP resulted in greater intake of neutral detergent fiber and ether extract compared with HTSBM. Cows

fed HTSBM and FP had lower sorting index for feed particles <4 mm than cows fed CON (1.029, 1.008, and 1.022). Feeding FP resulted in greater intake of feed particles <4 mm compared with HTSBM. Treatments containing HTSBM or FP tended to decrease organic matter digestibility (72.4, 71.2, and 71.1%), but no other effects were detected in digestibility of neutral detergent fiber, crude protein, or ether extract. No evidence for differences among treatments was detected in excretion of purine derivatives in milk and urine. Milk yield was greater in cows fed HTSBM or FP than in cows fed CON (28.0, 28.9, and 28.8 kg/d, respectively). Cows fed HTSBM or FP tended to have greater energy-corrected milk and protein yield compared with those fed CON. Milk protein concentration was greater in DDGS cows than those in the HTSBM group (3.45 and 3.40%, respectively). No differences were detected in milk fat yield and concentration, milk urea nitrogen, feed efficiency, or serum concentrations of urea and glucose. Overall, increasing dietary RUP by feeding HTSBM or FP improved intake of nutrients and milk yield without affecting feed efficiency. Altering digestible AA supply while maintaining similar dietary RUP had negligible effects on performance of cows.

Key words: amino acid, dietary protein, methionine, rumen undegradable protein

INTRODUCTION

In Brazil, soybean meal (SBM) products (heat treated or chemical treated) are the main ingredients providing RUP to the diets because corn gluten meal is scarce in certain regions and rumen-protected AA are relatively expensive. Furthermore, Brazilian federal regulations do not allow the inclusion of animal-derived products in diets of cattle to avoid any possible case of bovine spongiform encephalopathy (“mad cow disease”); thus, blood meal or fishmeal cannot be added to diets of ruminants (. Because a large portion of RUP

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in Brazilian diets is derived from SBM products, lactation diets are generally high in Lys and poor in Met. Dried distillers grains with soluble (DDGS) is a by-product from the corn ethanol industry that is rich in CP (especially RUP) and Met (Belyea et al., 2004; Liu, 2011). A commercially available high-protein (46% CP, of which 81.9% is RUP) corn DDGS containing protein derived from *Saccharomyces* spp. and from gluten (FP; FlexyPro, SJC Bioenergia) may be an alternative to commonly fed protein sources and other RUP supplements such as SBM and heat-treated soybean meal (HTSBM), respectively, in dairy cow diets.

Responses to increased RUP by lactating cows have varied in the literature, but positive effects in performance are observed, especially when treated SBM is used as the main RUP source in the diet (Santos et al., 1998; Bateman, 2005). Improvements in performance when supplementing RUP depend on intestinal digestibility and AA profile of RUP sources, as well as whether the increased flow of feed protein at the small intestine is offset, or not, by decreased microbial protein flow (Bateman, 2005). Feeding more RUP (+0.6% of diet DM) as expeller SBM resulted in improved feed efficiency and greater FCM and fat yield (Broderick and Reynal, 2009). Similarly, Zanton et al. (2013) reported marginal increases in milk yield (+1.37 kg/d) and FCM (+1.48 kg/d) with similar DMI when increasing dietary RUP from 7.1 to 7.9%. Recently, Brown and Bradford (2020) reported positive effects on milk and protein yield and feed efficiency in dairy cows fed diets with RUP sources (soy bypass protein combined with SBM or canola meal) replacing SBM.

Corn DDGS have relatively low Lys (1.87% of RUP) and high Met (2.40% of RUP) compared with typical protein sources such as SBM and HTSBM (Anderson et al., 2006). According to estimated duodenal Lys supply based on NRC (2001), partially replacing SBM (~30% of total SBM in diet) with FP would increase digestible Met supply from 44 to 50 g/d and digestible Lys supply from 190 to 194 g/d. Thus, using FP as a RUP source compared with HTSBM may improve the AA profile (i.e., a Lys-to-Met ratio closer to 3:1) and potentially increase productivity of dairy cows. However, although adding DDGS may improve digestible AA profile, DDGS in a diet may cause sorting by cows. Brown and Bradford (2020) fed a high-protein corn product in place of SBM in diets of dairy cows and reported greater CP concentration and lower CP intake as a percentage of total DMI compared with the other treatments, suggesting that cows sorted against the high-protein corn product. The authors speculated that this product imparted a flavor or odor that led cows to select against the high-protein corn diet (Brown and Bradford, 2020).

We hypothesized that higher dietary RUP would improve animal performance by increasing digestible AA supply and that cows fed FP would have greater milk protein yield compared with those fed HTSBM because of differences in AA profile. The objectives of this study were to evaluate the effect of increased RUP on nutrient intake, feed sorting index, nutrient total-tract apparent digestibility, excretion of purine derivatives (PD) in milk and urine, milk yield and composition, and serum concentrations of urea-N and glucose by including FP in diets of lactating cows and to determine whether the responses observed were due to digestible AA profile.

MATERIALS AND METHODS

This study was carried out in the Laboratório de Pesquisa em Bovinos de Leite (LPBL; Laboratory on Dairy Cattle Research, Pirassununga, Brazil) under the approval of the Ethics Committee on Animal Use from the School of Veterinary Medicine and Animal Sciences, University of São Paulo (protocol #5986020620).

Treatments and Experimental Design

Twenty-four Holstein cows (6 primiparous and 18 multiparous; 200 ± 40 DIM, 599 ± 78 kg of BW, and 30.0 ± 3.92 kg/d of milk yield at the start of the experiment) blocked according to parity, milk yield, and DIM were enrolled in a replicated 3×3 Latin square design experiment. Experimental periods lasted 21 d, with the first 14 d for treatment adaptation and 7 d for data collection. Treatments were (1) control (CON), a diet with 6.0% RUP containing 15.9% SBM as the main protein source; (2) HTSBM, a diet with 6.7% RUP containing 4.4% HTSBM (Soypass, Cargill Animal Health and Animal Nutrition) partially replacing SBM; and (3) high-protein DDGS, a diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn. Diets were formulated to achieve similar CP and NE_L . According to the manufacturer's information, FP is a type of DDGS, a by-product of corn ethanol production containing protein derived from *Saccharomyces* spp. and from gluten at a 20:80 ratio. According to the manufacturer's information, FP contains 46.0% CP, of which 81.9% is considered RUP, 3.5% Lys (% of CP), and 1.9% Met (% of CP). The RUP content of FP reported by the company was analyzed by a commercial laboratory using the method described in Krishnamoorthy et al. (1983). Wet chemistry analysis demonstrated that FP had 88.6% DM, 49.8% NDF, 28.2% ADF, 44.9% CP, 9.13% ether extract (EE), 4.61% Lys (% of CP), and 1.93 Met (% of CP). An in situ procedure was used to estimate RUP of HTSBM and FP (Chumpawadee

et al., 2011). Briefly, nylon bags (R1020, Ankom Technology Corp.) of 10×20 cm were filled with 5 g of the protein sources (25 mg/cm^2) and incubated in the rumen of 1 cannulated lactating dairy cow fed a corn silage-based diet. Bags were inserted into the rumen in reverse order for 48, 24, 16, 12, 8, 4, and 2 h, and all bags were removed simultaneously. Bag incubation residues were dried at 65°C for 72 h, weighed, and the residues were analyzed for CP. Bag residue CP was not corrected for microbial protein contamination. The RUP content in feeds was calculated according to Chumpawadee et al. (2011) considering a 5% passage rate (k_p). Average RUP values for HTSBM and FP were 73.4 and 81.8% CP, respectively. Soybean meal and HTSBM were chosen as treatments for comparison because they are the typical protein and bypass protein sources, respectively, fed on Brazilian farms and can be used as standard benchmarks for comparison with FP.

Animals were individually fed twice daily (0700 and 1300 h) in equal amounts, and the feeding rate was adjusted to allow refusals between 5 and 10% on an as-fed basis. Diets (Table 1) were formulated according to the NRC (2001) to meet or exceed nutrient estimate requirements of cows and were designed to have similar CP contents. Both HTSBM and DDGS diets were formulated to yield 10.0% (diet DM) RDP content. Cows were housed in a barn with individual pens (17 m^2 of area), sanded beds, fans, and free access to water. Samples of feed ingredients (Table 2) were collected during the last 7 d of each experimental period and analyzed for contents of DM (method 930.15; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), CP ($\text{N} \times 6.25$; Kjeldahl method 984.13; AOAC International, 2000), and EE (method 920.39; AOAC International, 2000). Neutral detergent fiber (Van Soest et al., 1991) was analyzed using α -amylase (TE-149 fiber analyzer; Tecnal Equipamentos para Laboratório Inc.), and ADF and lignin (method 973.18) were analyzed according to AOAC International (2000). Nonfiber carbohydrate was calculated according to Hall (2000). Feed ingredients were analyzed for contents of starch using an enzymatic degradation method (amyloglucosidase, Novozymes Latin America Ltda.) and absorbances measured by spectrophotometer (SBA-200, Celm) according to Hendrix (1993). Feed ingredient samples were pooled per period and shipped to a commercial laboratory (Laboratório CBO) for AA profiling (Table 3), except for tryptophan. Samples were submitted to HCl (6 N) hydrolysis and further derivatization with phenylisothiocyanate; AA were analyzed using HPLC and UV detection (White et al., 1986; Cohen and Strydom, 1988). Digestibility of RUP (%) in protein sources was considered 93.0 (NRC, 2001), 98.0 (Schumacher et

al., 2020), and 80.0 (NRC, 2001) for SBM, HTSBM, and FP, respectively.

Nutrient Intake, Sorting Index, and Apparent Digestibility

Feed offered and refusals were recorded daily to determine feed intake. Refusals were sampled during the last 7 d of each experimental period, pooled by cow per period, and frozen for further chemical analysis according to the methods described earlier. Samples of TMR and refusals were collected for 2 consecutive days during the collection period for determination of particle size distribution (Maulfair and Heinrichs, 2012) and sorting index (Silveira et al., 2007). Feed particles were stratified using the Penn State particle size separator to the following fractions: long (>19 mm), medium (19 to 8 mm), short (8 to 4 mm), and fine (<4 mm) particles. The sorting index was calculated using the following equations:

$$\text{Expected intake (kg/d)} = \text{intake (kg as-fed)/d} \\ \times P_{\text{TMR}} \text{ (kg/kg)},$$

$$\text{Observed intake (kg/d)} = [\text{offered (kg/d)} \\ \times P_{\text{TMR}} \text{ (kg/kg)}] - [\text{refusals (kg/d)} \\ \times P_{\text{Refusals}} \text{ (kg/kg)}],$$

$$\text{Sorting index} = \text{observed intake} \\ \text{(kg/d)/expected intake (kg/d)}.$$

The intake corresponding to each sieve was expressed as the percentage of the total estimated intake, where P_{TMR} is the TMR particle size and P_{refusals} is the particle size distribution of refusals. A sorting index of 1 indicates no sorting, a sorting index <1 indicates sorting against, and a sorting index >1 indicates sorting for particles on the particular screen.

Indigestible NDF (iNDF) contents in feeds, refusals, and feces were used to estimate fecal excretion of DM. Fecal samples ($n = 8$) were collected directly from the rectum of cows every 9 h during 3 consecutive days (d 15: 0600, 1500, and 0000 h; d 16: 0900 and 1800 h; d 17: 0300, 1200, and 2100 h) and pooled for further analyses. For the iNDF analysis, ground samples (2-mm) of feeds, refusals, and feces were placed in nonwoven fabric bags ($12 \mu\text{m}$ pore size, 5×5 cm at 20 mg DM/cm^2) and incubated in the rumen of 2 cannulated dry cows for 288 h (Huhtanen et al., 1994; Casali et al., 2008). After removal from the rumen, bags were washed in running tap water and dried, and the NDF content

Table 1. Ingredient and chemical composition (% of DM, unless otherwise noted), balance of digestible Met and Lys, and particle size distribution of diets

Item	Treatment ¹		
	CON	HTSBM	FP
Ingredient			
Corn silage ²	48.0	48.0	48.0
Ground corn	18.3	18.6	17.4
Citrus pulp	8.20	8.20	8.19
Soybean meal, 46% CP solvent	15.9	11.2	11.6
Whole raw soybean	6.47	6.47	6.47
Heat-treated soybean meal ³		4.40	
Dried distillers grains with solubles ⁴			5.34
Sodium bicarbonate	0.82	0.82	0.82
Mineral mixture ⁵	1.29	1.29	1.29
Limestone	0.73	0.73	0.73
Salt	0.26	0.26	0.26
Chemical composition			
DM	41.5	42.4	42.6
OM	92.4	92.5	92.4
Starch	29.3	29.5	28.6
CP	16.9	16.8	17.0
RDP ⁶	10.9	10.1	10.2
RUP ⁶	6.00	6.70	6.90
Ether extract	3.70	3.73	4.12
NDF	28.0	28.2	29.3
Forage NDF	26.5	26.5	26.5
NFC ⁷	42.5	39.4	35.1
NE _L , ⁶ Mcal/kg of DM	1.59	1.59	1.58
Ca	0.95	0.94	0.93
P	0.41	0.40	0.40
Mg	0.22	0.22	0.22
Cl	0.43	0.42	0.43
K	1.26	1.26	1.19
Na	0.43	0.43	0.43
S	0.20	0.20	0.22
Protein balance,⁸ g/d			
RDP	290	104	124
RUP	526	662	855
MP	449	537	712
MP requirements, ⁸ g/d	2,300	2,351	2,293
AA balance, g/d			
dLys			
Requirements ⁹	152	155	151
Supply ⁸	190	196	197
Balance	38	41	46
dMet			
Requirements ⁹	50.6	51.7	50.4
Supply ⁸	44	46	50
Balance	-6.6	-5.7	-0.4
Particle size distribution, % as-fed			
>19 mm	12.2	10.4	11.4
19-8 mm	36.9	38.8	35.2
8-4 mm	18.5	16.9	21.0
<4 mm	32.3	33.8	32.7

¹Control (CON): diet with 6.0% RUP containing 15.9% soybean meal (SBM) as the main protein source; heat-treated soybean meal (HTSBM): diet with 6.7% RUP containing 4.4% HTSBM (Soypass, Cargill Animal Health and Animal Nutrition) partially replacing SBM; and high protein DDGS (FP): diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn.

²Chemical (% of DM): 26.4% DM (as-fed), 95.9% OM, 40.8% NFC, 44.1% NDF, 28.6% ADF, 8.84% CP, 2.28% ether extract, and 26.4% starch.

³Soypass (Cargill Animal Health and Animal Nutrition).

⁴FlexyPro (SJC Bioenergia). Chemical (% of DM): 88.6% DM (as-fed), 97.0% OM, 25.0% NFC, 49.8% NDF, 28.2% ADF, 44.9% CP, 9.13% ether extract, and 5.70% starch.

⁵Contained per kg: 215 g Ca, 60 g P, 20 g S, 20 g Mg, 35 g K, 70 g Na, 15 mg Co, 700 mg Cu, 600 mg F, 10 mg Cr, 700 mg Fe, 40 mg I, 1,600 mg Mn, 20 mg Se, 2,500 mg Zn, 200,000 IU vitamin A, 50,000 IU vitamin D3, and 1,500 IU vitamin E.

⁶Estimated according to NRC (2001).

⁷Calculated according to Hall (2000).

⁸Calculated according to NRC (2001) using as inputs the outcomes of the current study (BW, milk yield and composition, and DMI).

⁹Requirements of digestible Met (dMet) and digestible Lys (dLys) were calculated as 2.2 and 6.6%, respectively, of MP requirements (NRC, 2001; Schwab et al., 2005; Lee et al., 2012).

Table 2. Chemical composition of feed ingredients (% of diet DM, unless otherwise stated)

Item	Soybean meal	Heat-treated soybean meal	DDGS ¹	Corn silage	Ground corn	Whole raw soybeans	Citrus pulp
DM, % as-fed	87.0	86.1	88.6	26.4	84.3	88.0	84.6
OM	93.6	96.0	97.0	96.0	98.3	95.0	94.2
NFC	22.9	19.4	2.50	40.8	69.8	9.16	66.1
NDF	20.0	24.9	49.8	44.1	33.6	31.5	17.8
ADF	8.97	12.8	28.2	28.6	12.2	15.9	13.2
CP	48.6	50.3	44.9	8.84	10.1	36.7	7.96
NDICP ²	6.35	10.8	22.3	1.69	2.13	11.0	2.08
ADICP ³	2.59	4.75	14.9	1.15	0.94	3.77	1.62
Ether extract	1.84	2.12	9.13	2.28	4.50	17.3	2.08
Lignin	0.68	0.55	0.55	5.20	0.54	0.61	0.53
Starch	5.45	3.54	5.70	25.0	78.7	4.46	9.36
Indigestible NDF	1.42	2.05	2.46	19.1	2.24	1.29	3.29

¹Dried distillers grains with solubles (FP; FlexyPro, SJC Bioenergia).

²Neutral detergent insoluble CP.

³Acid detergent insoluble CP.

was determined. Digestibility of DM and nutrients were calculated using following equations:

$$\text{DM digestibility (\%)} = 100 - \left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \right],$$

Nutrient digestibility (%) =

$$100 - \left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \times \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient intake}} \right) \right].$$

Excretion of Purine Derivatives

Urine samples (20 mL) were collected at the same time points of feces. Urine samples were diluted (1:4 ratio) into a sulfuric acid solution at 0.036 N to preserve PD (Chen and Gomes, 1992). Samples were stored frozen for total N, allantoin, uric acid, and creatinine analyses. Daily urine volume was estimated considering a daily creatinine excretion of 29 mg/kg of BW (Valadares et al., 1999). Body weights were measured (on 2 consecutive days) after the morning milking at the beginning of experiment and during the last 2 d of each

Table 3. Amino acid profile of feed ingredients and experimental diets

AA, % of DM	Corn silage ¹	Ground corn	Citrus pulp	Soybean meal	Whole raw soybean	HTSBM ²	FP	Treatment ³		
								CON	HTSBM	FP
Ala	0.751	0.690	0.324	2.23	1.67	2.26	3.24	0.976	0.973	1.047
Arg	0.697	0.593	0.605	4.70	3.46	4.75	2.78	1.464	1.453	1.405
Asp	0.370	0.711	0.669	6.28	4.65	6.32	3.45	1.662	1.647	1.570
Cys	0.054	0.140	0.032	0.664	0.535	0.671	0.771	0.195	0.193	0.206
Glu	0.849	1.78	0.507	8.97	6.68	9.079	7.540	2.633	2.616	2.634
Gly	0.316	0.377	0.302	2.13	1.64	2.16	1.83	0.690	0.686	0.692
His	0.131	0.410	0.194	1.99	1.47	1.97	1.97	0.565	0.560	0.581
Hyp	0.033	0.022	0.130	0.074	0.073	0.075	0.032	0.047	0.047	0.045
Ile	0.250	0.323	0.173	2.26	1.75	2.26	1.70	0.665	0.660	0.656
Leu	0.675	1.11	0.335	3.85	2.94	3.92	5.08	1.356	1.351	1.452
Lys	0.229	0.366	0.238	3.91	3.06	3.83	2.07	1.016	1.002	0.955
Met	0.065	0.162	0.000	0.548	0.409	0.564	0.866	0.175	0.174	0.196
Phe	0.272	0.442	0.216	2.51	1.92	2.55	2.12	0.752	0.748	0.754
Pro	0.490	0.862	0.972	2.55	1.94	2.59	3.79	1.004	1.000	1.089
Ser	0.207	0.442	0.238	2.60	1.88	2.66	2.31	0.735	0.731	0.743
Thr	0.250	0.302	0.151	1.99	1.48	2.03	1.84	0.600	0.597	0.610
Tyr	0.174	0.313	0.140	2.01	1.49	2.00	2.04	0.569	0.563	0.588
Val	0.338	0.453	0.227	2.31	1.83	2.31	2.33	0.748	0.743	0.770

¹Chemical (% of DM): 26.4% DM (as-fed), 95.9% OM, 40.8% NFC, 44.1% NDF, 28.6% ADF, 8.84% CP, 2.28% ether extract, and 26.4% starch.

²Heat-treated soybean meal (HTSBM; Soypass, Cargill Animal Health and Animal Nutrition).

³Control (CON): diet with 6.0% RUP containing 15.9% soybean meal (SBM) as the main protein source; heat-treated soybean meal (HTSBM): diet with 6.7% RUP containing 4.4% HTSBM (Soypass) partially replacing SBM; and high-protein DDGS (FP), diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn.

experimental period. Urine creatinine concentration was assessed using commercial kits (kinetic creatinine catalog no. K-067, Bioclin) and absorbances measured on spectrophotometer (SBA 200, Celm). Allantoin concentrations in urine and milk were assessed by a colorimetric method according to Chen and Gomes (1992), with absorbances measured on a microplate reader (Biochrom Asys UVM 340). Uric acid concentration in urine was analyzed using a commercial kit (uric acid stable liquid: catalog no. K-052; Bioclin). Total PD was calculated as the sum of allantoin excreted in milk and allantoin and uric acid excreted in urine (Orellana Boero et al., 2001).

Milk Yield and Composition and Serum Metabolites

Cows were milked twice daily (0600 and 1700 h), and production was recorded electronically (Alpro, DeLaval). Data from the last 7 d of milk yield were averaged and used for statistical analysis. Milk samples (300 mL) were collected for 3 consecutive days during each experimental period to assess concentrations of protein, fat, and lactose using a mid-infrared method (Lactoscan, Entelbra). Fat-corrected milk was calculated according to NRC (2001), where $4.0\% \text{ FCM} = 0.4 \times \text{milk (kg/d)} + 15 \times \text{fat (kg/d)}$. Milk samples were deproteinized with trichloroacetic acid solution (25%; 2:1 vol/vol; Shahani and Sommer, 1951) and stored at -20°C for allantoin and MUN analyses; MUN was determined using commercial kits (catalog no. K-056; Bioclin). Blood samples were collected from the tail veins on d 16 of each period, 4 h after the morning feeding. After clotting, blood samples were centrifuged (15 min, $2,000 \times g$ at room temperature), and the serum was harvested and stored at -20°C for further glucose and urea analyses. Blood urea N was calculated by multiplying the urea concentration by 0.4667. Blood glucose (catalog no. K082) and urea (catalog no K056) were measured using colorimetric commercial kits (Bioclin) and absorbances were measured on a semi-automatic biochemistry analyzer.

Statistical Analysis

Data were submitted to ANOVA using the PROC MIXED of SAS 9.4 (SAS Institute Inc.) according to the following model:

$$Y_{ijkl} = \mu + A_i + P_j + S_k + AS_{ik} + \alpha_{kl} + \varepsilon_{ijkl},$$

where $\alpha_{kl} \approx N(0, \sigma_c^2)$ and $\varepsilon_{ijkl} \approx N(0, \sigma_e^2)$, where N = Gaussian distribution; σ_c^2 = estimated variance associated with cows; and σ_e^2 = estimated residual variance,

Y_{ijkl} = observation on animal l , given treatment i , at period j , in square k ; μ = overall mean, A_i = fixed effect of the i th treatment ($i = 1$ to 3); P_j = fixed effect of the j th period ($j = 1$ to 3); S_k = fixed effect of the k th Latin square ($k = 1$ to 8); AS_{ik} = interaction fixed effect between treatment and Latin square; α_{kl} = random effect of animal within square ($l = 1$ to 24); and ε_{ijkl} = random error associated with each observation. Means were adjusted by LSMEANS, and degrees of freedom were calculated using the Kenward and Roger (1997) method. Treatment differences were evaluated by orthogonal contrasts (C), as follows: C1 = CON versus high-RUP sources (HTSBM + FP) and C2 = different digestible AA profile (HTSBM vs. FP). The significance level was set at $P \leq 0.05$ and tendencies were considered when $0.05 < P < 0.10$.

RESULTS

Increasing dietary RUP by using HTSBM or FP tended to increase ($P \leq 0.07$) intake (kg/d) of DM and OM and increased ($P \leq 0.03$) the intake of NDF, CP, and EE (Table 4). Dry matter intake was similar when comparing treatments with different digestible AA profile (HTSBM vs. FP). Cows fed FP tended to have greater ($P = 0.09$) NDF intake and had greater ($P < 0.01$) EE intake than cows in the HTSBM group. In terms of intake as a percent of BW, greater ($P \leq 0.01$) DM and NDF intakes were observed in cows fed high-RUP treatments, whereas NDF intake was greater in FP group compared with cows fed HTSBM. Sorting index for feeds with small particle size (between 8 and 4 mm and <4 mm) was lower ($P \leq 0.03$) in high-RUP treatments, whereas the FP group had greater ($P \leq 0.05$) sorting index for feeds with small particle size than the HTSBM group. No differences were detected for sorting index for feeds with particle size >19 mm and 19 to 8 mm. Organic matter digestibility tended to be lower ($P = 0.09$) in high-RUP treatments than in CON. No other treatment differences were detected for digestibility coefficients of DM, NDF, CP, and EE among groups.

Body weight tended to be greater ($P = 0.10$) in cows under high-RUP treatments than in CON, whereas FP cows had greater ($P = 0.04$) BW than those in HTSBM group (Table 5). Estimated urine output was similar between CON and treatments with high RUP, but urine output tended to be greater ($P = 0.10$) in cows fed FP compared with HTSBM. Similar PD excretion (allantoin in milk and urine, and uric acid in urine) was observed among treatment groups.

Yields of actual milk and lactose were greater in high-RUP treatments than in CON (Table 6). Energy-corrected milk and protein yield tended to increase (P

Table 4. Nutrient intake, sorting index, and total-tract apparent digestibility of mid-lactation cows fed different RUP sources partially replacing soybean meal in the diet

Item	Treatment ¹			SEM	P-value ²	
	CON	HTSBM	FP		C1	C2
Intake, kg/d						
DM	25.3	25.8	26.2	0.83	0.06	0.37
OM	23.2	23.6	24.1	0.81	0.07	0.22
NFC	12.0	12.0	11.8	0.40	0.67	0.34
NDF	7.14	7.35	7.69	0.237	<0.01	0.09
CP	4.27	4.37	4.51	0.143	0.03	0.13
Ether extract	0.942	0.968	1.04	0.0331	<0.01	<0.01
Intake, % of BW						
DM	3.84	3.96	3.98	0.146	0.01	0.66
NDF	1.10	1.13	1.17	0.041	<0.01	0.03
Sorting index ³						
>19 mm	0.920	0.938	0.935	0.0165	0.21	0.85
19–8 mm	0.977	0.941	0.968	0.0189	0.15	0.15
8–4 mm	1.005	0.978	0.998	0.0066	0.02	0.02
<4 mm	1.029	1.008	1.022	0.0069	0.03	0.05
Digestibility coefficient, %						
DM	70.2	70.0	68.7	0.75	0.18	0.11
OM	72.4	71.2	71.1	0.91	0.09	0.96
NDF	36.0	37.3	36.6	0.95	0.36	0.61
CP	69.9	69.6	68.7	0.97	0.44	0.42
Ether extract	80.4	79.3	79.7	1.54	0.45	0.76

¹Control (CON): diet with 6.0% RUP containing 15.9% soybean meal (SBM) as the main protein source; heat-treated soybean meal (HTSBM): diet with 6.7% RUP containing 4.4% HTSBM (Soypass) partially replacing SBM; and high-protein DDGS (FP), diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn.

²Orthogonal contrasts: C1 = CON vs. high-RUP sources (HTSBM + FP); C2 = different digestible AA profile (HTSBM vs. FP).

³No sorting = 1, values <1 indicates sorting against, and values >1 indicates sorting for particles on the particular particle size range. Sorting index was calculated according to Silveira et al. (2007).

≤ 0.10) when feeding high RUP treatments. No differences in milk and solids yield were observed between HTSBM and FP groups. Increasing dietary RUP had no effect on milk concentration of fat, protein, or lactose, but milk protein concentration was greater ($P = 0.01$) in cows fed FP than in cows fed HTSBM. Milk urea nitrogen concentration, feed efficiency, and serum

concentrations of urea-N and glucose were not affected by treatments.

DISCUSSION

We hypothesized that increasing dietary RUP by using either HTSBM or FP would improve performance,

Table 5. Purine derivative excretion of mid-lactation cows fed different RUP sources partially replacing soybean meal in the diet

Item	Treatment ¹			SEM	P-value ²	
	CON	HTSBM	FP		C1	C2
BW, kg	650	651	656	13.2	0.10	0.04
Estimated urine, L/d	19.6	18.8	21.0	1.28	0.80	0.10
Purine derivatives, mmol/d						
Milk allantoin	23.8	20.3	26.5	3.83	0.83	0.14
Urine allantoin	246	272	259	23.9	0.51	0.69
Urine uric acid	118	126	127	9.6	0.35	0.93
Total	388	418	444	33.1	0.28	0.57

¹Control (CON): diet with 6.0% RUP containing 15.9% soybean meal (SBM) as the main protein source; heat-treated soybean meal (HTSBM): diet with 6.7% RUP containing 4.4% HTSBM (Soypass) partially replacing SBM; and high-protein DDGS (FP), diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn.

²Orthogonal contrasts: C1 = CON vs. high-RUP sources (HTSBM + FP); C2 = different digestible AA profile (HTSBM vs. FP).

Table 6. Milk yield and composition, feed efficiency, and blood metabolites of mid-lactation cows fed different RUP sources partially replacing soybean meal in the diet

Item	Treatment ¹			SEM	<i>P</i> -value ²	
	CON	HTSBM	FP		C1	C2
Yield, kg/d						
Milk	28.0	28.9	28.8	0.57	0.05	0.82
4% FCM ³	29.7	30.7	30.5	0.60	0.12	0.70
ECM ⁴	32.5	33.5	33.4	0.59	0.10	0.87
Fat	1.24	1.28	1.26	0.030	0.22	0.69
Protein	0.96	0.98	0.99	0.019	0.07	0.44
Lactose	1.44	1.49	1.48	0.028	0.04	0.80
Composition, %						
Fat	4.45	4.47	4.43	0.106	0.95	0.64
Protein	3.43	3.40	3.45	0.019	0.76	0.01
Lactose	5.14	5.17	5.15	0.031	0.29	0.47
MUN, mg/dL	9.80	9.24	9.19	0.406	0.23	0.90
Efficiency						
Milk yield ÷ DMI	1.12	1.14	1.12	0.040	0.46	0.17
FCM ÷ DMI	1.29	1.32	1.28	0.038	0.67	0.20
ECM ÷ DMI	1.30	1.32	1.30	0.039	0.64	0.26
Blood metabolites, mg/dL						
Urea-N	18.7	18.8	18.3	0.47	0.71	0.38
Glucose	57.1	56.3	57.7	0.99	0.90	0.24

¹Control (CON): diet with 6.0% RUP containing 15.9% soybean meal (SBM) as the main protein source; heat-treated soybean meal (HTSBM): diet with 6.7% RUP containing 4.4% HTSBM (Soypass) partially replacing SBM; and high-protein DDGS (FP), diet with 6.9% RUP containing 5.34% FP (FlexyPro, SJC Bioenergia) partially replacing SBM and ground corn.

²Orthogonal contrasts: C1 = CON vs. high-RUP sources (HTSBM + FP); C2 = different digestible AA profile (HTSBM vs. FP).

³FCM was calculated according to NRC (2001), where 4.0% FCM = 0.4 × milk (kg/d) + 15 × fat (kg/d).

⁴ECM was calculated according to NRC (2001), where ECM (kg) = [0.327 × milk (kg)] + [12.95 × fat (kg)] + [7.65 × protein (kg)].

and altering digestible AA profile (i.e., Lys-to-Met ratio closer to 3:1) would result in enhanced milk protein yield when comparing HTSBM and FP treatments. It is important to highlight that the model for estimating protein degradability in the rumen used by NRC (2001) contains inaccuracies, as pointed out in Tedeschi et al. (2015) and in NASEM (2021). For instance, Broderick et al. (2010) meta-analyzed data of 32 studies and reported that NRC (2001) underpredicted RDP (i.e., overpredicted RUP) by 22% compared with observed omasal values. Inaccuracies in RDP and RUP estimates by NRC (2001) support positive responses in milk yield and DMI observed in literature when feeding more RUP to cows. Although in the current experiment, RDP and RUP amounts were fed in excess according to NRC (2001) estimates, feeding protein sources rich in protein fractions not degraded in the rumen increased milk yield without affecting total-tract digestibility of CP and excretion of PD. Improvements in performance when feeding more RUP may be observed if the increased flow of feed protein at the small intestine is not offset by decreased microbial protein flow (Bateman, 2005). Data of different studies, collectively, suggest that a minimum of 10% RDP is required to not impair DMI and microbial protein supply in dairy cows (Rey-

nal and Broderick, 2005; Broderick and Reynal, 2009). Note that the current NASEM (2021) recommends a minimum diet RDP of 10%, which was used as the RDP threshold level in diet formulations for the current study. Improved performance of cows fed HTSBM and FP is associated with greater intake of DM, NDF, and CP, and supported by no differences in feed efficiency. The greater sorting for shorter feed particles observed in HTSBM and FP over CON might be a potential factor for differences in milk yield. An increase in sorting index for shorter feed particles when feeding FP contrasts with speculations by Brown and Bradford (2020) regarding flavor or odor imparted during the production of a type of DDGS.

Increased dietary RUP promoted feed intake of cows but tended to reduce total-tract apparent digestibility of OM. Greater DMI results in an increase in feed passage rate and less time available for degradation in the rumen and digestion throughout the gut. The reasons for the marginal increase in DMI when feeding RUP sources are not clear, based on the data reported in this study. Aligning with the results observed in this study, increasing dietary RUP by adding HTSBM or DDGS has been shown to increase DMI and milk yield (Janicek et al., 2008; Benchaar et al., 2013; Martins et

al., 2019). The replacement of urea (0.78% of diet DM) and SBM (10.3% of diet DM) with HTSBM (at 4.47% of diet DM) in corn silage- and ground corn-based diets increased DMI and milk yield of mid-lactation cows (Martins et al., 2019). Replacing SBM (13.2% of diet DM) with different doses of DDGS (10 to 30% of diet DM) has linearly increased DMI and milk yield of cows fed diets with varied CP contents (Benchaar et al., 2013). Feeding corn DDGS (at either 10 or 20% of diet DM) replacing SBM and ground corn improved milk (+1.9 kg/d) and protein yields and feed efficiency in dairy cows (Anderson et al., 2006). Other studies have reported either no effects on milk yield and DMI (de Lima et al., 2013; Maxin et al., 2013; Castillo-Lopez et al., 2014) or tendencies for decreased DMI (Anderson et al., 2006; Morris et al., 2018) when replacing SBM with DDGS. Earlier studies have not shown evidence for effects of HTSBM on DMI and milk yield (Ahrar and Schingoethe, 1979; Driver et al., 1990). We can ask whether changing RUP dietary levels from 6.0% to 6.70 to 6.90% is sufficient to alter milk production; we did show that altering RUP levels from 5.15 to 6.30% increased milk production of cows (Martins et al., 2019). Janicek et al. (2008) altered dietary RUP from 6.87 to 7.49%, leading cows to produce 3.2 kg/d more milk. These studies demonstrated that relatively small differences in RUP levels can promote differences in milk yield even larger than those observed in the current experiment. Those studies, however, did not report values or balances of estimated MP supply to determine whether diets were sufficient in RUP. It is worth mentioning that the cows used in the current study are less efficient in terms of milk yield for every kilogram of DM consumed compared with the cows of studies cited above (Janicek et al., 2008; Benchaar et al., 2013).

Most studies evaluating dietary inclusion of DDGS in diets have reported either an increase or no effect on DMI and milk yield (Paz et al., 2013; Castillo-Lopez et al., 2014; Foth et al., 2015; Ranathunga et al., 2018; Testroet et al., 2018), whereas few studies have reported negative effects of DDGS on performance of dairy cows (Morris et al., 2018). The reasons why Morris et al. (2018) found lowered performance in cows fed DDGS are not clear but might be related to the high inclusion rate of DDGS and consequently large amounts of PUFA in the diet, dietary RDP level (not reported), or AA balancing. For example, the control diet had a Lys:Met ratio of 4.00, whereas the DDGS diet had a Lys:Met ratio of 2.11. In addition, cows fed DDGS had a lower Lys plasma concentration than CON cows (Morris et al., 2018). The studies cited above used higher DDGS inclusion rates ($\geq 9.7\%$ of diet DM) than those in the present study, suggesting that even small changes in

dietary protein profile (i.e., degradability and AA content) may improve productivity of cows. For instance, increasing the dietary content of RUP by less than 1% and Met by 0.021% resulted in 0.80 kg/d more milk produced by cows fed FP compared with CON. Some studies feeding more RUP by changing protein sources or supplementing rumen-protected AA, however, have reported no effects in performance of dairy cows (Castillo-Lopez et al., 2014; Lee et al., 2015; Ranathunga et al., 2018). The reasons for the lack of responses could be related to inaccuracies in MP estimation (Broderick et al., 2010; NASEM, 2021) and imbalances in MP and digestible AA, which are often not reported in studies with protein and AA nutrition.

Because one objective of this study was to evaluate the effects of altering the digestible AA profile of diets with similar RUP, the inclusion level of FP was based on dietary RUP of HTSBM treatment. Low inclusion rates of FP may also avoid potential negative effects of DDGS in milk fat when fed at levels $>10\%$ of diet DM as reported in some studies (Zanton et al., 2013; Morris et al., 2018). High inclusions of DDGS may increase the dietary concentration of 18:2 fatty acid (as % of long-chain fatty acids), resulting in greater milk *trans*-10, *cis*-12 18:2 fatty acid concentration (Morris et al., 2018). It is well known that *trans*-10, *cis*-12 18:2 down-regulates milk fat synthesis (Harvatine et al., 2018). We speculate that the relatively low inclusion of DDGS in the current study did not promote an increase in blood *trans*-10, *cis*-12 concentration large enough to impair milk fat synthesis. Castillo-Lopez et al. (2014) fed DDGS at 9.7% of diet DM and did not find differences in milk fat concentration and production (Castillo-Lopez et al., 2014). It is important to highlight that different responses of altering protein nutrition in performance (i.e., milk yield and feed efficiency) of cows might be observed in short-term (3- to 4-wk periods, Latin square) or in long-term (randomized complete block design) study designs; however, interaction effects between experimental design (changeover vs. continuous design) and treatments were not observed for yields of milk fat and protein (Zanton, 2016).

It is important to note that even though HTSBM or DDGS increased CP intake without altering CP digestibility, treatments did not affect MUN or BUN, supporting the trend for greater milk protein yield over the CON group. In contrast to most studies demonstrating that feeding DDGS at $\leq 20\%$ has no effect on milk protein percentage (Anderson et al., 2006; Kleinschmit et al., 2007; Schingoethe et al., 2009), feeding DDGS resulted in greater milk protein concentration compared with HTSBM treatment in this study. Replacing soybean products with DDGS has also increased milk protein concentration and yield without affecting DMI

in early lactation cows (Mjoun et al., 2010). Differences in milk protein concentration between cows fed HTSBM and FP are negligible because no differences were detected in milk protein yield when comparing both treatments. Studies that attempted to replace bypass protein sources with DDGS are scarce in the literature (Mjoun et al., 2010; Brown and Bradford, 2020). Feeding a novel high-protein corn product (56.1% CP; co-product from ethanol production) in place of SBM and expeller SBM, authors reported lower ECM, fat, and protein yield, and DM digestibility (Brown and Bradford, 2020). These authors related the negative effects of the high-protein corn product to formation of Maillard products during drying (Brown and Bradford, 2020).

As we expected, all diets were apparently excessive for Lys and poor in Met. Note that NRC (2001) considers the RUP fraction of feeds to have the same AA profile as the feed CP, and we did not observe treatment differences in PD excretion (a proxy of rumen microbial protein synthesis). Earlier studies have proposed an approximately 3:1 ratio of Lys to Met in MP to improve milk protein synthesis (Armentano et al., 1997; Rulquin et al., 2006; Wang et al., 2010; Osorio et al., 2013). In the current study, the FP treatment had the lowest Lys-to-Met ratio (3.98), whereas other treatments had values around 4.3. Furthermore, the balance of digestible Met (supply – requirements) was greater in FP than in other treatments. Differences in Lys-to-Met ratio between treatments were expected to be greater than we observed; the Lys content in DDGS reported by the manufacturer (3.5% of CP) was lower than that observed (4.61% of CP) in the current study. In addition, the Met content in diet might have been underestimated because some sulfur AA can be lost during the HCl hydrolysis used for AA profile determination. Cysteine and methionine are unstable AA and can be degraded during acid hydrolysis because of the reaction of sulfur with residual oxygen by the formation of methionine sulfoxide or sulfone and cysteine sulfenic, sulfenic, or sulfonic acid (Lamp et al., 2018). One caveat is that we did not measure the intestinal digestibility of RUP fraction of HTSBM and FP to accurately estimate MP supply. According to NRC (2001) calculations, treatments containing sources rich in RUP (HTSBM and FP) would provide greater MP balance than CON, which could be associated with increases in milk yield. According to PD excretion, FP dietary inclusion did not alter microbial protein supply in the current study, which is in line with previous studies in the literature (Janicek et al., 2008; Maxin et al., 2013).

In summary, we demonstrated that our hypothesis—that increasing dietary RUP using either HTSBM or FP would improve performance of dairy cows—was

confirmed by increased milk yield, which is supported by increased DMI and higher balances of digestible AA. The expected improvement in protein yield when altering digestible AA profile using FP to replace soybean products was not evident in this study. Feeding DDGS in place of soybean products had minor effects on nutrient intake and performance of cows, demonstrating that DDGS can be a valuable substitute for HTSBM, depending on market price fluctuations and product availability. Furthermore, replacing corn starch and SBM products with DDGS has been economically beneficial to dairy producers when included up to 20% of diet DM (Ranathunga et al., 2010, 2018). In the current study, FP (at 5.34% FP) replaced 0.90% ground corn and 4.3% (of diet DM) SBM in the CON diet. In other words, for every 1,000 kg of diet DM, around 1 kg of ground corn and 4 kg of SBM were replaced by 5 kg of DDGS. According to ERS (2022), in 2020–2021, the average cost of corn was \$178/t, that of SBM was \$230/t, and that of distillers dried grains was \$198/t. Thus, if the performance is maintained, replacing SBM with DDGS in diets of dairy cows seems economically favorable.

CONCLUSIONS

Partially replacing SBM with either HTSBM (4.40% of diet DM) or a high-protein corn DDGS (5.34% of diet DM) to increase dietary RUP improved feed intake and milk yield without affecting DM digestibility or PD excretion in mid-lactation cows. Compared with HTSBM, feeding the high-protein corn DDGS had negligible effects on milk protein concentration as no differences in milk yield and milk protein concentration were observed. This study demonstrated that the high-protein corn DDGS (at 5.34% of diet DM) can be a suitable substitute for HTSBM.

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