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Extruded soybean meal increased feed intake and milk production in dairy cows

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ABSTRACT

The objective of this study was to assess the effects of 2 extruded soybean meals (ESBM) processed at 2 extruder temperatures, 149°C (LTM) and 171°C (HTM), on performance, nutrient digestibility, milk fatty acid and plasma amino acid profiles, and rumen fermentation in lactating dairy cows. Nine multiparous Holstein cows were included in a replicated 3 × 3 Latin square design experiment with three 28-d periods. The control diet contained 13% solvent-extracted soybean meal (SSBM; 53.5% crude protein with 74.1% ruminal degradability and 1.8% fat), which was replaced with equivalent amount (dry matter basis) of LTM (46.8%, 59.8%, and 10.0%) or HTM (46.9%, 41.1%, and 10.9%, respectively) ESBM in the 2 experimental diets (LTM and HTM, respectively). The diets met or exceeded the nutrient requirements of the cows for net energy of lactation and metabolizable protein. The 2 ESBM diets increased dry matter intake and milk yield compared with SSBM. Feed efficiency and milk composition were not affected by treatment. Milk protein yield tended to be increased by ESBM compared with SSBM. Milk urea N and urinary urea N excretions were increased by the ESBM diets compared with SSBM. Concentration of fatty acids with chain length of up to C17 and total saturated fatty acids in milk fat were generally decreased and that of C18 and total mono- and polyunsaturated fatty acids was increased by the ESBM diets compared with SSBM. Blood plasma concentrations of His, Leu, and Val were increased by HTM compared with LTM and SSBM. Plasma concentration of Met was decreased, whereas that of carnosine was increased by the ESBM diets. Treatments had no effect on rumen fermentation, but the proportion of *Fibrobacter* spp. in whole ruminal contents was increased by HTM compared with SSBM and LTM. Overall, data from this crossover experiment suggest that substituting SSBM with ESBM in the diet has a positive effect on feed intake and milk yield in dairy cows.
Key words: extruded soybean meal, feed intake, milk fatty acid, dairy cow

INTRODUCTION

Microbial protein synthesized in the rumen and feed RUP are the main sources of AA for dairy cows (NRC, 2001), and their AA composition is becoming increasingly important when cows are fed diets supplying MP close to or below their requirements. We have demonstrated, for example, that AA such as His may become limiting in dairy cows fed MP below NRC (2001) requirements, partially due to the relatively lower concentration of His in microbial protein, compared with other EAA such as Met (Lee et al., 2012). Even if MP requirements are met, milk production and/or milk protein concentration may be increased by key EAA, through supplementation of the diet with synthetic rumen-protected AA, or digestible RUP from dietary origin (Broderick et al., 2009; Patton et al., 2014). This may be particularly true with alfalfa silage-based diets, which may be high in CP but still supply inadequate amounts of MP and EAA (Broderick et al., 1990; Dhiman et al., 1993). Several heat-treated soybean meal (**SBM**) products have been developed with the goal of providing a digestible RUP source in the diet. The production responses, however, have been variable (Broderick, 1986; Broderick et al., 1990; Socha, 1991; Flis and Wattiaux, 2005). Most of the commercial heat-treated SBM products have fat content of 1.2 to 2.2% (Amino Plus, AGP, Omaha, NE; Soy Pass, Ligno Tech USA, Overland Park, KS) up to 6.6% (SoyPLUS, West Central Cooperative, Ralston, IA; Soy Best, Grain States Soya Inc., West Point, NE). In a preliminary experiment, we analyzed extruded SBM (**ESBM**) and found a linear increase in its RUP content (determined in situ) with increasing the extruder temperature from 149°C to 160°C and 171°C (Isenberg et al., 2012). The ESBM contained around 10% fat, which may provide

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additional energy for high-producing dairy cows and may also favorably modify milk FA composition, assuming it does not negatively affect ruminal fermentation and fiber digestibility.

Therefore, the current experiment was conducted to test the effects of 2 ESBM on performance, nutrient digestibility, milk FA and plasma AA profiles, rumen fermentation variables, and bacterial and archaeal composition of ruminal contents in dairy cows. We hypothesized that, when substituting solvent-extracted SBM (**SSBM**) on an equal-weight basis, ESBM will increase plasma concentrations of key EAA, C18 unsaturated FA in milk, and milk yield or milk protein yield (or both) in dairy cows fed a corn silage-based diet.

MATERIALS AND METHODS

All procedures carried out in the experiment were approved by the Animal Care and Use Committee at The Pennsylvania State University.

Animals and Experimental Design

The experiment was a replicated 3 × 3 Latin square design balanced for residual effects and was conducted in the tie-stall barn of The Pennsylvania State University's Dairy Teaching and Research Center. Nine multiparous lactating Holstein cows averaging (±SD): 141 (±31.0) DIM, 41.5 (±4.68) kg/d of milk yield, and 650 (±54.7) kg of BW at the beginning of the study, were grouped into 3 squares based on DIM, milk yield, and parity. Six cows (2 squares) were fitted with 10-cm (internal diameter) soft plastic ruminal cannulas (Bar Diamond Inc., Parma, ID). Each experimental period lasted 28 d, with 18 to 21 d of adaptation to the diets, followed by 10 (DMI, BW, and milk yield and composition) or 7 (all other variables) days of data and sample collection. Cows were randomly assigned to 1 of 3 treatment diets (Table 1), which contained 13% of (DM basis) (1) SSBM (Cargill Inc., Roaring Spring, PA), (2) low-temperature (149°C) extruded SBM (**LTM**), or (3) high-temperature (171°C) extruded SBM (**HTM**). The extruded SBM were produced by Fabin Bros. Farms (Indiana, PA). Composition of the SBM used in the experiment is shown in Table 2. Diets were formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows yielding 41 kg of milk/d with 3.50% milk fat and 3.04% true protein at 25.5 kg/d of DMI and 638 kg of BW. The 2 ESBM diets were supplemented with 0.35% of urea (DM basis, replacing corn grain) to achieve a similar N concentration to the SSBM diet, and because of the lower RDP content of the 2 ESBM compared with SSBM (see Table 1). The 3 diets were mixed using a Kuhn Knight model 3142 Reel Auggie Mixer Wagon (Kuhn Knight Inc., Brodhead, WI) and were fed once daily (0630 h) as TMR to achieve about 5 to 10% refusals. All cows had free access to drinking water, were milked twice daily (0500 and 1700 h), and received rbST (Posilac, Elanco Co., Greenfield, IN; 500 mg, i.m.) at 14-d intervals (i.e., at the beginning and in the middle of each experimental period).

Sampling and Measurements

Individual feed intake (on as-fed basis) and milk yield of the cows were recorded daily throughout the experiment. Cow BW was also recorded daily for the entire experiment using AfiFarm 3.04E scale system (S.A.E. Afikim, Rehovot, Israel) while cows exited the milk parlor. Total mixed ration and refusals from each diet were sampled twice weekly, and samples were composited (on an equal weight basis) by week and diet. Samples of individual forages (i.e., corn silage, alfalfa haylage, and grass hay) and concentrate feeds were collected weekly. Forages were composited by experimental period, whereas one composite sample for the entire experiment was prepared for each concentrate feed ingredient. All samples were stored at −20°C, dried for DM determination at 65°C for 48 h in a forced-air oven, and ground with a Wiley Mill (1-mm screen; Thomas Scientific, Swedesboro, NJ) for further analyses. Dry matter intake was computed from the as-fed TMR intake using the DM content of the weekly composited TMR and refusals samples. Composite samples of individual feed ingredients were analyzed by wet chemistry methods for CP, degradable protein (SSBM and ESBM only; according to Krishnamoorthy et al., 1983), NDF, ADF, fat, ash, Ca, P, and estimated NFC and NEL by Cumberland Valley Analytical Services (Maugansville, MD; analytical methods are available from CVAS, 2015). The analyzed composition of the feed ingredients and their inclusion in the TMR was used to compute the CP, NDF, ADF, fat, Ca, and P concentration of the diets (Table 1). Concentration of RDP, RUP, NE_L, NFC and MP, protein fractions, and AA balances were estimated using NRC (2001) based on actual DMI, milk yield, milk composition, and BW of the cows during the experiment. Composite TMR samples were analyzed for starch according to Bach Knudsen (1997) and indigestible NDF (**iNDF**) as described by Lee et al. (2012). Samples of the 3 SBM (i.e., SSBM, LTM, and HTM) were also analyzed for AA (AOAC International, 2006, method no. 982.30 E) at the University of Missouri–Columbia's Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

Table 1. Ingredients and chemical composition of the diets and protein and AA balance during the experiment

Item	Diet[1]		
	SSBM	LTM	HTM
Ingredient, % of DM			
Corn silage[2]	40.0	40.0	40.0
Alfalfa haylage[3]	20.0	20.0	20.0
Grass hay[4]	5.0	5.0	5.0
Cottonseed, hulls	5.0	5.0	5.0
Corn grain, ground	9.00	8.65	8.65
SBM, solvent-extracted	13.0	—	—
SBM, extruded	—	13.0	13.0
Urea	—	0.35	0.35
Molasses[5]	5.0	5.0	5.0
Mineral/vitamin premix[6]	2.8	2.8	2.8
Salt	0.2	0.2	0.2
Composition, % of DM			
CP[7]	16.0	16.0	16.0
RDP[8]	10.0	9.7	8.8
RUP[8]	5.9	6.2	7.1
NDF[7]	33.4	33.5	33.6
ADF[7]	22.5	22.5	22.5
Fat[7]	3.09	4.23	4.35
Starch[9]	20.5	20.4	20.4
NE_L[8] Mcal/kg	1.49	1.51	1.51
NE_L balance,[8] Mcal/d	3.9	5.5	5.1
NFC[10]	44.0	42.7	42.5
Ca[7]	0.81	0.81	0.81
P[7]	0.32	0.33	0.33
Protein and AA balance,[8,11] g/d			
MP			
Requirements	2,588	2,723	2,735
Supply	2,845	3,091	3,200
Balance	256	368	465
RDP and RUP			
RDP supply	2,669	2,754	2,494
RDP balance	124	42	−212
RUP supply	1,592	1,760	2,006
RUP balance	306	437	546
dMet			
Requirements[12]	57	60	60
Supply	49	53	53
Balance	−8	−7	−7
dLys			
Requirements[12]	171	180	181
Supply	183	197	195
Balance	12	17	14
dHis			
Requirements[12]	57	60	60
Supply	58	63	65
Balance	1	3	5

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.
[2]Corn silage was 40.9% DM and (DM basis): 36.7% NDF and 8.1% CP.
[3]Alfalfa haylage was 45.2% DM and (DM basis): 42.4% NDF and 18.6% CP.
[4]Grass hay contained (DM basis): 74.0% NDF and 7.1% CP.
[5]Molasses (Westway Feed Products, Tomball, TX) contained (DM basis) 3.9% CP and 66% total sugar.
[6]The premix (Cargill Animal Nutrition, Cargill Inc., Roaring Spring, PA) contained (%, as-is basis) trace mineral mix, 0.86; MgO (56% Mg), 8.0; NaCl, 6.4; vitamin ADE premix (Cargill Animal Nutrition, Cargill Inc.), 0.48; limestone, 37.2; selenium premix (Cargill Animal Nutrition, Cargill Inc.), 0.07; and dry corn distillers grains with solubles, 46.7. Ca, 14.1%; P, 0.39%; Mg, 4.59%; K, 0.44%; S, 0.39%; Se, 6.91 mg/kg; Cu, 362 mg/kg; Zn, 1,085 mg/kg; Fe, 186 mg/kg, vitamin A, 276,717 IU/kg; vitamin D, 75,000 IU/kg; and vitamin E, 1,983 IU/kg.
[7]Values calculated using the chemical analysis (Cumberland Valley Analytical Services Inc., Maugansville, MD) of individual feed ingredients of the diet.
[8]All values were estimated based on NRC (2001) using actual DMI, milk yield, milk composition, and BW of the cows throughout the trial.
[9]Values determined using an enzymatic colorimetric method (Bach Knudsen, 1997) on TMR composites.
[10]Estimated by NRC (2001).
[11]dLys, dMet, dHis = digestible Lys, Met, and His, respectively. Due to rounding, balance may not exactly match requirements and supply.
[12]Requirements of dLys and dMet were calculated as 6.6 and 2.2% (respectively) of MP requirements (Schwab et al., 2005). Requirements of dHis were assumed as 2.2% of MP requirements (Lee et al., 2012).

Table 2. Crude protein, fat, and AA concentration of the soybean meals (SBM) fed in the experiment

	Soybean meal[1]		
Item	SSBM	LTM	HTM
CP,[2] % DM	53.5	46.8	46.9
RDP,[3] % of CP	74.1	59.8	41.1
RUP, % of CP	25.9	40.2	58.9
Crude fat, % DM	1.82	10.0	10.9
EAA, % CP			
Arg	7.17	6.91	6.90
His	2.49	2.38	2.40
Ile	4.45	4.35	4.29
Leu	7.96	7.66	7.76
Lys	6.47	6.43	6.22
Met	1.44	1.42	1.45
Phe	5.19	5.01	5.08
Thr	3.85	3.80	3.85
Trp	1.46	1.33	1.41
Val	4.58	4.44	4.40
Total EAA	45.1	43.7	43.8
NEAA, % CP			
Ala	4.18	4.28	4.37
Asp	11.2	10.8	11.0
Cys	1.32	1.35	1.38
Glu	17.8	17.0	17.2
Gly	3.87	4.12	4.09
Pro	4.95	4.99	4.97
Ser	4.43	4.44	4.46
Tyr	3.52	3.48	3.50
Total NEAA	51.3	50.5	51.0

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]N × 6.25.

[3]Estimated using the *Streptomyces griseus* protease method (Krishnamoorthy et al., 1983) by Cumberland Valley Analytical Services (Maugansville, MD).

During the last week of each experimental period, 8 spot fecal and urine samples (approximately 500 g and 300 mL/sample, respectively) were collected in 3 consecutive days at intervals staggered in time to cover a 24-h period. Fecal samples were oven-dried at 65°C for 48 h, ground through a 1-mm sieve, composited per cow and experimental period, and then analyzed for starch (Bach Knudsen, 1997) and DM, OM, CP, NDF, ADF, and iNDF as described in Lee et al. (2012). Total-tract apparent digestibility of DM, OM, NDF, ADF, CP, and starch was estimated using iNDF as an internal digestibility marker. Urine samples were processed and analyzed for allantoin, uric acid, creatinine, urea-N, and total N as described by Lee et al. (2012). Daily urine volume was calculated using creatinine as a marker, assuming a creatinine excretion rate of 29 mg/kg of BW (Hristov et al., 2011), and was used to estimate urinary N and purine derivative excretions.

Blood samples were collected from the tail vein or artery into heparinized vacutainers at 2 and 4 h after feeding on d 24 and 25 of each experimental period. Blood plasma was separated and processed (Lee et al., 2012) for analysis of urea N (Stanbio Urea Nitrogen Kit 0580, Stanbio Laboratory Inc., Boerne, TX), AA, and the dipeptides carnosine and anserine at the University of Missouri–Columbia's Agricultural Experiment Station Chemical Laboratory following the procedures of Deyl et al. (1986) and Fekkes (1996).

Milk samples were collected from 2 consecutive milkings (p.m. and a.m.) in 3 separate days (d 20 and 21, 24 and 25, and 27 and 28) during each experimental period. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol and submitted to Dairy One Laboratory (Pennsylvania DHIA, University Park, PA) for analysis of fat, true protein, lactose, and MUN using infrared spectroscopy (MilkoScan 4000, Foss Electric, Hillerød, Denmark). Evening and morning milk samples were analyzed separately so milk component concentration could be weighed for p.m and a.m. milk yields. A separate unpreserved milk sample was collected during wk 4 of each experimental period and stored at −20°C for FA composition analysis as described elsewhere (Hristov et al., 2010).

Samples of whole ruminal contents were collected from the cannulated cows at 2, 4, and 6 h after feeding on d 27 and 28 of each experimental period. These

samples were collected, processed, and analyzed for pH, ammonia, VFA, and bacteria and archaeal order and genus composition as described in Hristov et al. (2013a), with the exception that samples for microbial composition were stored at −80°C before analysis.

A subexperiment was conducted to determine in situ ruminal degradability of CP of the 3 SBM products (i.e., SSBM, LTM, and HTM). The experimental procedures were as described by Lee et al. (2012), except 2 cows were used for the rumen incubation; 7 g of SBM per bag was incubated in the rumen of the cows in triplicate for 0, 2, 4, 8, 24, and 48 h; and bags with in situ residues were dried at 65°C for 48 h. The cows used in the in situ experiment were fed (% of DM): corn silage, 43; grass hay, 7.9; ground corn grain, 9.9; canola meal, 8.0; whole roasted soybeans, 7.9; SoyPLUS (West Central Cooperative, Ralston, IA), 6.9; bakery byproduct meal, 6.0; cottonseed hulls, 3.8; molasses, 3.4; and a mineral and vitamin premix, 3.2. Aliquots of each in situ bag residue was pulverized using a Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) and analyzed for N using elemental analyzer (Costech ECS 4010 C/N/S, Costech Analytical Technologies Inc., Valencia, CA) to calculate CP (N × 6.25). Rumen-degraded protein and RUP values of the SSBM, LTM, and HTM products were estimated using NRC (2001) equations.

Another subexperiment was conducted to evaluate the heat stability of SSBM Lys. A single sample of the SSBM used in the main experiment was heated in a forced-air oven to temperatures ranging from 100°C to 200°C in 20°C intervals for 1 h and was analyzed for Lys as described above.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (2003, SAS Institute Inc., Cary, NC). Milk yield, DMI, and BW data for the last 10 d of each experimental period were averaged and the average values were used in the statistical analysis. Feed efficiency (milk yield ÷ DMI) was estimated based on the average milk yield and DMI data. Milk composition data were also averaged per cow, and experimental period and the average values were used in the statistical analysis. The statistical model included treatment and experimental period. Square and cow within square were random effects and all others were fixed.

Data from the in situ experiment were analyzed using the NLMIXED procedure of SAS. Degradability data for replicated bags incubated in each cow at each time point were averaged and the average values were used in the statistical analysis. In situ degradability parameters [soluble N, fraction a; potentially degradable fraction, fraction b; rate of degradation (disappearance) of the degradable fraction, c; and effective degradability in the rumen, **ED**] were estimated according to the models of Ørskov and McDonald (1979) using dummy variable technique for treatment comparisons and contrasts (PROC NLMIXED; adjusted R^2 was 0.98 to 0.99). A passage rate of 0.06/h was used to estimate ED.

When the main effect of treatment was significant, means were separated by pairwise *t*-test (pdiff option of PROC MIXED). Statistical differences were considered significant at $P \leq 0.05$ and a trend at $0.05 \leq P \leq 0.10$. Data in tables are presented as least squares means.

RESULTS

The nutrient composition of the 3 diets was similar (Table 1) with the exception that fat concentration was higher with the LTM and HTM diets compared with SSBM because of the higher fat content of ESBM versus SSBM (around 10 vs. 1.8%). The 3 diets provided NE_L, MP, RDP (except the HTM diet), and RUP in excess of the estimated (NRC, 2001) cow requirements. The diets were about 14, 12, and 12% (SSBM, LTM, and HTM, respectively) deficient in digestible Met, according to Schwab et al. (2005). All 3 diets supplied adequate amounts of digestible Lys and His, according Schwab et al. (2005) and Lee et al. (2012), respectively.

The CP concentration of the SSBM was higher compared with the ESBM as a result of its lower fat content (Table 2). As expected, the proportion of RDP in CP was higher for SSBM, whereas HTM had the highest proportion of RUP. Concentrations of Arg, His, and Trp were about 4 to 6% lower for ESBM compared with SSBM. Concentration of Lys was also about 4% lower for HTM compared with SSBM. Overall, concentrations of most EAA, except Met and Thr, were slightly decreased in ESBM versus SSBM. Of the NEAA, Gly concentration was about 6% higher for ESBM versus SSBM.

Dry matter intake, milk and lactose yields, and MUN were increased ($P \leq 0.05$) by LTM and HTM, compared with SSBM (Table 3). Milk protein yield tended ($P = 0.09$) to be higher for the ESBM diets versus SSBM. Concentration of plasma urea N was increased ($P = 0.03$) for LTM compared with SSBM. No differences among treatments were observed ($P > 0.10$) for feed efficiency, milk fat, protein, and lactose concentrations and milk fat, ECM, and milk NE_L yields.

Intake of all nutrients during the digestibility measurement periods was ($P \leq 0.05$) or tended ($P = 0.06$ for OM and starch) to be increased by the ESBM diets compared with SSBM (Table 4). Apparent total-tract digestibilities of DM, OM, and ADF were slightly

Table 3. Dry matter intake and milk production variables in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Item	SSBM	LTM	HTM	SEM[2]	*P*-value
DMI, kg/d	26.9[b]	28.2[a]	28.1[a]	1.49	0.05
Milk yield, kg/d	37.5[b]	40.6[a]	40.9[a]	2.21	0.01
Milk yield ÷ DMI, kg/kg	1.43	1.44	1.49	0.062	0.28
Milk fat, %	3.60	3.38	3.42	0.16	0.23
Milk fat, kg/d	1.34	1.37	1.40	0.065	0.68
Milk true protein, %	2.95	2.90	2.86	0.053	0.23
Milk true protein, kg/d	1.11	1.17	1.17	0.053	0.09[3]
Milk lactose, %	4.71	4.72	4.71	0.062	0.93
Milk lactose, kg/d	1.77[b]	1.92[a]	1.93[a]	0.10	0.04
MUN, mg/dL	13.1[b]	14.7[a]	14.8[a]	0.47	<0.01
ECM,[4] kg/d	34.5	36.2	36.5	1.64	0.25
ECM ÷ DMI, kg/kg	1.29	1.29	1.31	0.046	0.74
Milk NE_L,[5] Mcal/d	25.7	27.0	27.2	1.22	0.26
BW, kg	625	645	631	24.4	0.81
PUN,[6] mg/dL	10.3[b]	12.6[a]	11.7[ab]	0.76	0.03

[a,b]Means with different letter superscripts differ at $P < 0.05$.

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table; n = 26 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]SSBM vs. LTM, $P < 0.05$.

[4]Energy-corrected milk (kg/d) = kg of milk × [(38.3 × % fat × 10 + 24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7) ÷ 3,140] (Sjaunja et al., 1990).

[5]Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).

[6]Plasma urea N.

Table 4. Nutrient intake and total-tract apparent digestibility in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Item	SSBM	LTM	HTM	SEM[2]	*P*-value
Nutrient intake,[3] kg/d					
DM	26.9[b]	28.2[a]	28.0[a]	1.15	0.05
OM	25.1	26.3	26.1	1.08	0.06
NDF	8.98[b]	9.45[a]	9.42[a]	0.38	0.03
ADF	6.05[b]	6.35[a]	6.31[a]	0.26	0.05
CP	4.30[b]	4.51[a]	4.49[a]	0.18	0.05
Starch	5.50	5.77	5.72	0.23	0.06
Apparent digestibility, %					
DM	64.0[ab]	63.4[b]	64.7[a]	0.32	0.01[4]
OM	65.3[ab]	64.7[b]	65.7[a]	0.33	0.03[5]
NDF	39.3	38.2	39.4	0.80	0.45
ADF	37.4[ab]	36.7[b]	39.1[a]	0.81	0.04[6]
CP	64.7	63.0	64.1	1.14	0.50
Starch	97.5[a]	96.9[b]	96.8[b]	0.24	0.01

[a,b]Means with different letter superscripts differ at $P < 0.05$.

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table; n = 26 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Intake data during the digestibility measurement periods.

[4]SSBM vs. LTM, $P = 0.09$; SSBM vs. HTM, $P = 0.10$.

[5]SSBM vs. LTM, $P = 0.08$.

[6]SSBM vs. HTM, $P = 0.07$.

Table 5. Milk FA composition (g/100 g of total FA) in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Fatty acid	SSBM	LTM	HTM	SEM[2]	*P*-value
4:0	3.99	4.14	4.04	0.143	0.39
6:0	2.35	2.30	2.25	0.079	0.38
8:0	1.41[a]	1.31[b]	1.28[b]	0.052	0.02
10:0	3.40[a]	2.92[b]	2.86[b]	0.148	<0.001
12:0	3.84[a]	3.16[b]	3.09[b]	0.151	<0.001
14:0	10.9[a]	9.97[b]	9.89[b]	0.197	<0.001
14:1	1.16[a]	0.95[b]	1.00[b]	0.091	<0.01
15:0	0.90[a]	0.75[b]	0.78[b]	0.025	<0.01
16:0	28.4[a]	24.7[b]	23.8[c]	0.62	<0.001
16:1	1.74[a]	1.33[b]	1.38[b]	0.129	<0.001
17:0	0.48[a]	0.44[b]	0.43[b]	0.011	<0.01
18:0	8.05[b]	9.86[a]	9.82[a]	0.381	<0.001
18:1, *trans*-4	0.02[c]	0.03[b]	0.03[a]	0.001	<0.001
18:1, *trans*-5	0.02[b]	0.03[a]	0.03[a]	0.001	<0.001
18:1, *trans*-6–8	0.30[b]	0.44[a]	0.47[a]	0.016	<0.001
18:1, *trans*-9	0.24[c]	0.35[b]	0.37[a]	0.010	<0.001
18:1, *trans*-10	0.50[b]	0.74[a]	0.88[a]	0.093	<0.01
18:1, *trans*-11	0.93[c]	1.57[b]	1.75[a]	0.071	<0.001
18:1, *trans*-10 + *trans*-11	1.42[c]	2.31[b]	2.63[a]	0.127	<0.001
18:1, *cis*-9	20.9[b]	22.6[a]	22.5[a]	0.796	0.04
18:1, *cis*-11	1.09[b]	1.27[a]	1.30[a]	0.058	<0.001
18:2, *cis*-9,*cis*-12	2.71[c]	3.54[b]	4.22[a]	0.090	<0.001
18:3	0.38[c]	0.46[b]	0.51[a]	0.013	<0.001
CLA-*cis*-9,*trans*-11	0.42[b]	0.64[a]	0.73[a]	0.052	<0.001
CLA-*trans*-10,*cis*-12	ND	ND	ND	0.001	0.28
20:0	0.10	0.11	0.11	0.006	0.11
Others	5.33[b]	5.66[a]	5.82[a]	0.118	<0.01
Total *trans* FA	2.46[c]	3.82[b]	4.22[a]	0.162	<0.001
Σ SFA	63.9[a]	59.6[b]	58.2[c]	1.16	<0.001
Σ MUFA	26.6[b]	29.4[a]	29.9[a]	0.98	<0.001
Σ PUFA	3.09[c]	4.00[b]	4.73[a]	0.100	<0.001

[a–c]Means with different letter superscripts differ at $P < 0.05$.

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table; n = 26 (n represents number of observations used in the statistical analysis); ND = not detected. Data are presented as LSM.

higher ($P \leq 0.04$) for HTM versus LTM. Digestibility of starch was about 1% lower ($P = 0.01$) for the ESBM diets versus SSBM.

Compared with SSBM, the ESBM diets had decreased ($P \leq 0.02$) concentrations of 8:0, 10:0, 12:0, 14:0, 14:1, 15:0, 16:0, 16:1, and 17:0 FA in milk fat (Table 5). Concentration of 16:0 was decreased ($P < 0.05$) by HTM compared with LTM. On the other hand, concentrations of 18:0, *trans*- and *cis*-18:1, *cis*-9,*cis*-12, 18:2, 18:3, *cis*-9,*trans*-11 CLA, and total *trans* FA were increased ($P \leq 0.04$) by the ESBM diets, compared with SSBM. Overall, the ESBM diets resulted in lower concentrations of saturated FA and greater concentrations of MUFA and PUFA ($P < 0.001$) compared with SSBM.

Blood plasma concentrations of His, Leu, Val, and 1-methylhistidine (**1-MH**) were increased ($P \leq 0.03$) by HTM compared with SSBM and LTM (Table 6). Concentration of Met was decreased ($P = 0.05$) and that of carnosine was increased ($P = 0.02$) by the ESBM diets compared with SSBM. Plasma Ile and Tau concentrations tended to be increased ($P \leq 0.07$) by the ESBM diets compared with SSBM, whereas plasma Thr tended to be lower ($P = 0.07$) for the ESBM diets. Plasma Lys concentration was not affected by diet. Concentration of Pro was increased ($P = 0.02$) by HTM versus SSBM. Concentration of 3-methylhistidine (**3-MH**) tended to be lower ($P = 0.10$) for HTM compared with SSBM and LTM.

During the fecal and urine sampling periods, N intake was higher ($P = 0.05$) and secretion of milk true-protein N tended to be increased ($P = 0.10$) for the ESBM diets compared with SSBM (Table 7). Urinary urea N (UUN) excretion was increased ($P = 0.01$), and as a result, UUN excretion as a proportion of N intake tended to be higher ($P = 0.08$) for the ESBM diets ver-

Table 6. Blood plasma AA concentration (μM) in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Item	SSBM	LTM	HTM	SEM[2]	*P*-value
EAA					
Arg	66.3	69.6	69.5	2.98	0.37
His	39.9[b]	43.3[b]	50.0[a]	2.62	<0.01
Ile	100	105	112	5.36	0.07
Leu	115[b]	121[b]	133[a]	7.34	0.02
Lys	66.6	66.9	64.4	2.19	0.58
Met	18.4[b]	16.7[a]	16.4[a]	1.14	0.05
Phe	39.3	39.5	40.5	1.45	0.66
Thr	90.3	85.0	79.4	5.08	0.07
Trp	67.5	66.6	67.4	2.67	0.97
Val	190[b]	196[b]	215[a]	12.2	0.03
NEAA					
Ala	197	202	208	9.33	0.55
Asn	33.8	33.7	34.3	0.97	0.90
Asp	6.30	6.15	7.19	0.75	0.32
Cit	65.9	73.7	71.0	6.45	0.17
Cys	0.22	0.30	0.27	0.08	0.84
Gln	208	203	198	5.34	0.40
Glu	44.3	45.1	44.8	2.18	0.81
Gly	241	243	254	15.3	0.35
Orn	36.7	37.7	38.4	1.67	0.59
Pro	69.0[b]	73.4[ab]	79.8[a]	3.05	0.02
Ser	78.6	78.6	83.1	2.63	0.13
Tau	30.4	31.8	35.4	2.26	0.06
Tyr	40.8	40.6	40.6	2.26	0.99
1-MH[4]	4.99[b]	4.87[b]	7.88[a]	0.58	<0.001
3-MH[4]	3.39	3.50	3.08	0.38	0.10
Anserine	0.03	0.09	ND[3]	0.05	0.15
Carnosine	10.3[b]	12.0[a]	12.7[a]	0.68	0.02
EAA	794	811	850	37.8	0.12
NEAA	914	922	948	29.2	0.34
EAA+NEAA	1,708	1,733	1,798	60.9	0.14

[a,b]Means with different letter superscripts differ at $P < 0.05$.

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table; n = 26 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Not detected.

[4]1- and 3-methylhistidine.

sus SSBM. Estimated total N losses with urine, feces, and milk, urine output, and urinary excretion of purine derivatives were not affected by diet.

Ruminal ammonia concentration was numerically increased ($P = 0.15$) for the ESBM diets compared with SSBM, whereas all other rumen fermentation parameters were not affected by diet (Table 8). Bacterial and archaeal order and genus compositions of whole ruminal contents are presented in Table 9. *Fibrobacterales*, *Fibrobacter*, and *Barnesiella* were increased ($P \leq 0.04$) by HTM compared with SSBM and LTM. *Rhodocyclales* and *Azospira* tended to be increased ($P = 0.08$), and *Alistipes* was decreased ($P < 0.01$), respectively, by the ESBM diets versus SSBM. *Dorea* was increased ($P = 0.04$) by HTM compared with LTM. The order or genus composition of methanogenic archaea was not affected by treatment.

The in situ N degradability, or disappearance, data for the 3 SBM are shown in Figure 1. The rate of N degradability was about 1.8 to 2.6 times greater ($P \leq 0.002$) for SSBM versus both LTM and HTM. The rate of N degradation was not different between the 2 extruded meals. Similarly, ED of N was greater ($P < 0.001$) for SSBM versus ESBM and ED of LTM was also greater ($P < 0.001$) than that of HTM.

Data for the heat-stability test of Lys in SSBM are shown in Figure 2. Although not analyzed statistically (n = 1), Lys concentration in SSBM protein appears to have started to markedly decrease with heating temperatures >160°C for 1 h: an average of 6.61 for temperatures between 0 (unheated) and 140°C versus 6.50, 6.37, and 5.78% of the total analyzed AA for 160, 180, and 200°C, respectively.

Table 7. Milk N secretion, urinary and fecal N excretion, and urinary purine-derivative (PD) excretion in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Item	SSBM	LTM	HTM	SEM[2]	*P*-value
N intake, g/d	688[b]	722[a]	718[a]	29.4	0.05
N secretion and excretion, g/d					
Milk TPN[3]	174	184	183	7.2	0.10
Urinary N	153	176	175	14.4	0.26
Urinary urea N (UUN), g/d	93[b]	117[a]	116[a]	7.9	0.01
UUN ÷ total urinary N, %	61.8	69.5	67.3	4.68	0.51
Fecal N	245	246	235	11.7	0.66
Total excreta N	398	423	413	21.1	0.48
Total N in excreta and milk	572	609	598	24.6	0.24
As proportion of N intake %					
Milk TPN	25.5	25.5	25.6	0.84	0.97
Urine N	21.9	23.1	22.9	1.72	0.88
UUN	13.5	15.8	15.6	0.78	0.08
Fecal N	35.8	34.4	33.5	1.22	0.29
Total N in excreta and milk	83.5	84.2	83.9	2.11	0.96
Urine output, kg/d	19.7	21.6	21.5	1.72	0.48
Urinary PD excretion, mmol/d					
Allantoin	548	514	529	37.8	0.71
Uric acid	63.8	60.2	62.4	6.17	0.78
Total PD	611	574	591	42.1	0.71

[a,b]Means with different letter superscripts differ at $P < 0.05$.

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table; n = 26 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[3]Milk true protein N (milk true protein ÷ 6.38).

Table 8. Rumen fermentation variables in dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[1]				
Item	SSBM	LTM	HTM	SEM[2]	*P*-value
pH	5.93	6.04	6.02	0.106	0.55
Ammonia, m*M*	2.40	2.76	2.82	0.180	0.15
VFA, m*M*					
Total	137.2	138.9	140.3	5.29	0.86
Acetate	83.5	85.8	87.0	2.63	0.55
Propionate	28.7	27.6	29.2	2.11	0.85
Butyrate	19.2	19.3	17.8	1.35	0.46
Isobutyrate	1.01	0.98	0.98	0.058	0.82
Valerate	2.74	2.80	2.69	0.128	0.71
Isovalerate	2.21	2.09	2.24	0.212	0.77
As % of total					
Acetate	61.0	61.7	61.7	0.84	0.34
Propionate	20.6	19.7	20.5	1.00	0.84
Butyrate	13.9	13.8	12.5	0.77	0.32
Isobutyrate	0.76	0.72	0.74	0.038	0.74
Valerate	2.01	2.02	1.93	0.047	0.35
Isovalerate	1.69	1.55	1.72	0.152	0.73
Acetate:propionate	3.05	3.18	3.06	0.181	0.86

[1]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[2]Largest SEM published in table. Rumen pH, ammonia, and VFA data, n = 17 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

Table 9. Bacterial and archaeal order and genus composition (as % of total isolates[1]) in whole ruminal contents of dairy cows fed diets containing solvent-extracted or extruded soybean meals (SBM)

	Diet[2]				
Item	SSBM	LTM	HTM	SEM[3]	*P*-value
Bacteria order					
Clostridiales	55.1	55.5	55.1	1.56	0.50
Bacteroidales	26.5	25.9	24.1	1.41	0.49
Bifidobacteriales	3.49	3.49	2.87	1.519	0.50
Fibrobacterales	2.01[b]	2.33[b]	3.48[a]	0.377	0.04
Erysipelotrichales	1.96	2.34	2.47	0.360	0.22
Spirochaetales	1.51	1.73	1.76	0.292	0.63
Lactobacillales	1.60	1.51	1.36	0.157	0.46
Rhodocyclales	1.04	1.77	1.68	0.218	0.08
Chromatiales	1.30	1.10	1.19	0.179	0.76
Micrococcales	1.07	1.12	1.29	0.224	0.52
Mycoplasmatales	1.04	1.01	0.69	0.216	0.45
Bacteria genus					
Prevotella	16.9	16.9	14.6	1.45	0.43
Ruminococcus	7.96	6.53	6.89	0.94	0.48
Clostridium	6.07	6.04	5.66	0.28	0.55
Butyrivibrio	4.98	5.28	5.99	0.39	0.26
Acetitomaculum	5.11	5.03	5.42	0.34	0.54
Coprococcus	4.44	4.48	4.98	0.85	0.56
Blautia	4.65	4.71	4.44	0.46	0.89
Barnesiella	4.04[b]	3.94[b]	4.44[a]	0.35	<0.01
Pseudobutyrivibrio	3.36	3.48	3.74	0.36	0.69
Saccharofermentans	3.02	3.40	3.11	0.22	0.24
Bifidobacterium	3.49	3.49	2.87	1.52	0.50
Dorea	2.85	2.49	3.58	0.26	0.09[4]
Succiniclasticum	2.80	2.71	2.34	0.32	0.62
Fibrobacter	2.01[b]	2.33[b]	3.48[a]	0.38	0.04
Fecalibacterium	1.66	2.21	1.80	0.24	0.13
Treponema	1.49	1.71	1.74	0.29	0.61
Alistipes	1.98[a]	1.34[b]	0.95[b]	0.22	<0.01
Catenibacterium	1.35	1.66	1.61	0.19	0.43
Azospira	1.04	1.77	1.68	0.22	0.08
Streptococcus	1.56	1.47	1.32	0.16	0.47
Bacteroides	1.30	1.19	1.41	0.19	0.19
Flavonifractor	1.06	1.28	1.39	0.20	0.45
Xylanibacter	1.24	1.24	1.17	0.12	0.83
Roseburia	1.24	1.02	1.48	0.21	0.34
Thioalkalibacter	1.29	1.09	1.19	0.18	0.76
Dermatophilus	1.07	1.12	1.29	0.22	0.52
Sarcina	1.02	1.16	0.78	0.18	0.40
Mycoplasma	1.04	1.01	0.69	0.22	0.45
Archaea order					
Methanobacteriales	99.9	99.9	99.9	0.04	0.80
Methanomicrobiales	0.05	0.05	0.04	0.03	0.92
Archaea genus					
Methanobrevibacter	91.2	90.6	90.3	0.72	0.68
Methanosphaera	8.74	9.25	9.65	0.71	0.67
Methanomicrobium	0.05	0.05	0.04	0.03	0.92

[a,b]Means with different letter superscripts differ at $P < 0.05$.

[1]The percentage represents the percentage of the total sequences analyzed within the sample.

[2]SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C.

[3]Largest SEM published in table; n = 17 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

[4]HTM versus LTM ($P = 0.04$).

DISCUSSION

As a result of exposure to a higher temperature during the extrusion process (known to reduce rumen degradability of CP; Solanas et al., 2008), the HTM had the highest RUP concentration followed by LTM and SSBM. In a pilot study leading to the current experiment (Isenberg et al., 2012), we observed a linear increase ($R^2 = 0.99$) in RUP content, estimated from in situ protein degradability data, of ESBM with extru-

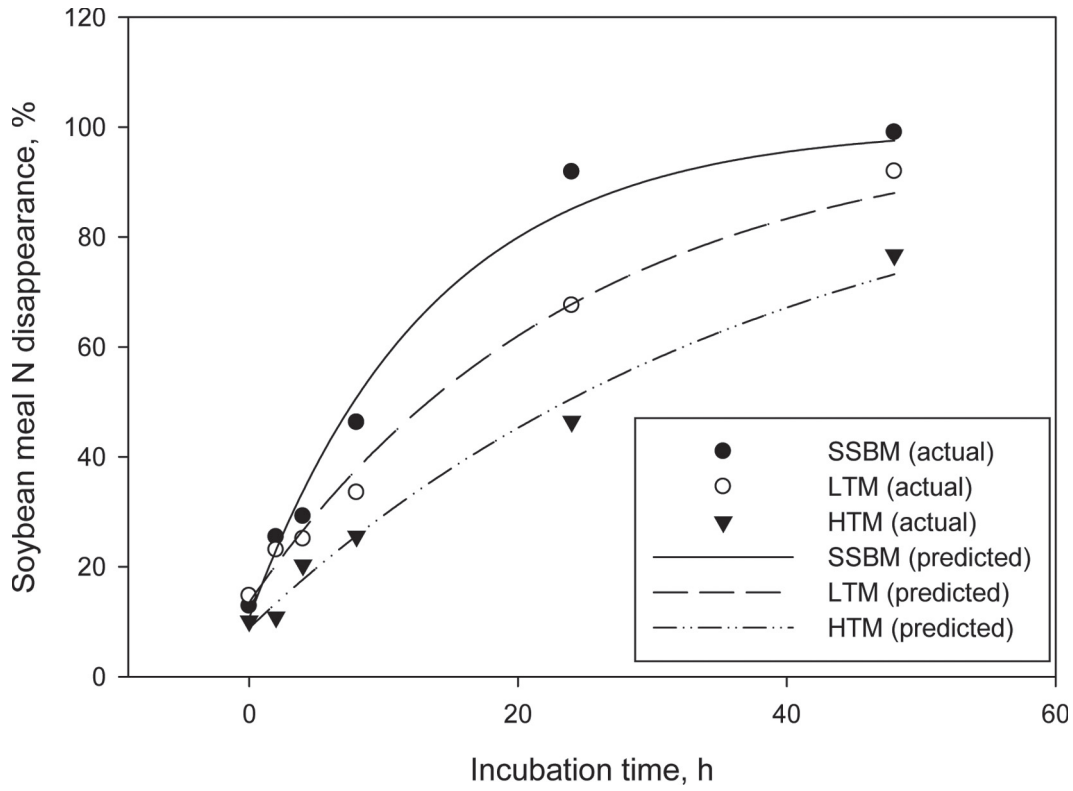

Figure 1. In situ disappearance of nitrogen from the soybean meals (SBM) used in the experiment. SSBM = solvent-extracted SBM; LTM = SBM extruded at 149°C; HTM = SBM extruded at 171°C. Rate of disappearance (%/h): $6.8^{a} \pm 0.83$, $3.7^{b} \pm 0.76$, and $2.6^{b} \pm 0.74$ (SSBM, LTM, and HTM, respectively); Effective degradability (%; estimated at 6%/h rate of passage): $60.4^{a} \pm 1.41$, $48.2^{b} \pm 1.64$, and $36.2^{c} \pm 1.44$, respectively. Means with different letters (a–c) differ at $P \leq 0.009$.

sion temperatures of 149, 160, and 171°C. It is worth noticing that in that study, meal output was decreased by about 13% by increasing the extrusion temperature from 149 to 171°C.

The observed lower concentration of some EAA (e.g., Arg, His, and Phe) in the ESBM diets compared with SSBM, and the lower Lys concentration with the higher extrusion temperature (i.e., HTM), is in agreement with several other studies (Sahlu et al., 1984; Broderick, 1986) on heat-treated SBM. A similar decrease in Lys concentration of SBM with increasing the heating temperature above 160°C was also observed in the pilot study reported in Figure 2. These effects have been attributed to formation of Maillard products and cross links among AA (Björck and Asp, 1983; Csapó et al., 2008).

The effect of the ESBM on DMI and milk production in the current experiment was remarkable. The diets were not deficient in NE_L and supplied MP in excess of NRC (2001) requirements. The estimated balance of key EAA was also similar between SSBM and the ESBM diets. Based on the current NRC (2001) requirements, the increased milk yield with ESBM cannot be attributed to increased RUP or NE_L (due to higher fat content) concentration or supply. Thus, it appears that the increased milk yield with LTM and HTM was primarily due to the increased DMI with these diets compared with SSBM. This is supported by the similar feed efficiency among the diets (although some numerical increase appeared to occur with the ESBM diets).

Earlier studies with heat-treated SBM reported increased milk yield and feed efficiency in high- (but not low-) producing dairy cows (Sahlu et al., 1984). A series of experiments with expeller SBM (reaching maximum processing temperature of 163°C) showed variable responses in milk yield compared with SSBM, in spite of much greater concentration of RUP than SSBM (Broderick, 1986; Broderick et al., 1990). Those experiments suggested that protein from expeller SBM can replace greater amounts of SSBM and maintain the same level of production. Socha (1991) summarized 9 experiments with heat-treated SBM conducted between 1979 and 1990 and reported increased DMI in 3 and increased milk yield in 8 of the experiments. Milk fat and protein concentrations appeared to be decreased in 6 experiments. Overall, milk yield was increased by 1.8 kg/d at

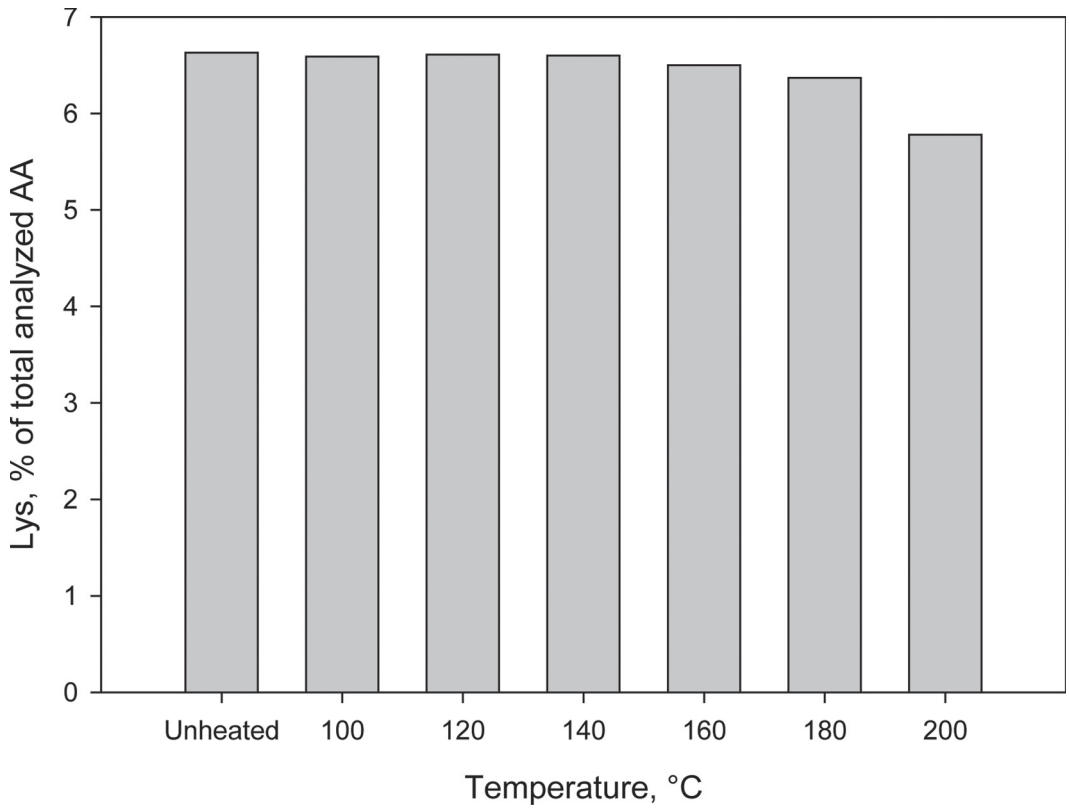

Figure 2. Concentration of Lys in solvent-extracted soybean meal heated for 60 min at different temperatures (a single sample was analyzed at each temperature; SD = 0.28).

similar or slightly lower (−0.1 kg/d) DMI. Similar to data from the current experiment, Flis and Wattiaux (2005) reported increased DMI and milk production with expeller SBM supplemented at about 10% over NRC (2001) requirements for RUP. In a more recent study by Broderick et al. (2009), expeller SBM had no effect on milk yield (a 0.8 kg/d numerical increase occurred), but feed efficiency, milk fat concentration, and 3.5% FCM milk yield were increased compared with the control, SSBM, diet. Others, however, reported no or variable effects of heat-treated SBM on feed intake or lactational performance of dairy cows (Ipharraguerre et al., 2005; Awawdeh et al., 2007).

The general trend in milk FA composition observed in the current experiment was for decreased concentration of FA with chain length up to C17 and increased concentration of long-chain, C18 FA, with ESBM. This resulted in increased concentrations of CLA, total *trans* FA, MUFA, and PUFA, but decreased total SFA. It is established that long-chain FA in milk fat are predominantly derived from dietary sources (Palmquist and Jenkins, 1980), and therefore, the results with ESBM in the current experiment are not surprising. A similar increase in milk C18 FA from cows fed heat-treated soybean meal, whole-soybeans, or soybean oil is commonly reported in the literature (Socha, 1991; Sauer et al., 1998; AlZahal et al., 2008; Chen et al., 2008).

Plasma concentration of several key EAA, such as His, Leu, and Val, increased as a result of feeding ESBM, particularly HTM, in the current experiment. Concentration of Met, however, considered first or second (along with Lys) limiting AA in typical North American dairy diets (NRC, 2001), was decreased by ESBM. In the experiments of Broderick (1986), plasma concentrations of EAA were similar between diets containing expeller SBM versus SSBM. Similar to our current data, however, Met concentration decreased in the experiment with the highest inclusion rate of expeller SBM. Awawdeh et al. (2007) also did not report differences in plasma AA concentrations between cows fed diets with SSBM or expeller SBM. In that experiment, the AA composition of SSBM and expeller SBM was also not different and digestibility of RUP from expeller SBM, tested in chicken, was not different from that of SSBM. Based on the hypothesis that EAA will not accumulate in blood plasma unless supplied in excess of requirement (Almquist, 1954; Broderick et al., 1974) and the lower plasma concentration of Met in cows fed

ESBM (which also had higher milk yield, compared with the control), it could be concluded that Met was perhaps the first limiting AA in the current experiment. This is in agreement with the data by Broderick (1986), who also found decreased plasma Met and total sulfur AA with expeller SBM in one of their experiments. The greater concentrations of His, Leu, and Val with ESBM in the current experiment can be explained with increased supply of these AA with RUP, compared with SSBM. Carnosine is a dipeptide containing β-Ala and His and muscle carnosine is considered an endogenous source of His in ruminants or monogastric animals (see discussion in Giallongo et al., 2015). Its increased concentration with the ESBM diets is in agreement with the increased plasma His concentrations. The increase in plasma 1-MH with HTM was interesting and consistent among experimental animals (SE = 0.57 μM). Plasma 1-MH may result from the metabolism of the dipeptide anserine contained in meat and fish, and its excretion in urine was suggested as a marker for meat intake in animals or humans (Myint et al., 2000). It is not clear why HTM triggered such a large response in plasma 1-HM concentration, particularly with the lack of increase in 3-MH, considered a marker for muscle catabolism. In fact, a trend was observed for decreased 3-MH with HTM versus LTM ($P = 0.12$) or the control ($P = 0.04$).

Broderick (1986) suggested that heating of SBM could result in decreased Lys availability. In one of his experiments with expeller SBM (in which the inclusion rate was the highest; 22% on DM basis), replacing SSBM with expeller SBM resulted in decreased plasma Lys concentration and the appearance of an unidentified postlysine peak (presumed to be derived from Lys during the expeller processing). In the pilot study conducted along with the current experiment, concentration of Lys in SSBM heated at temperatures of up to 140°C (which was the processing temperature reached for LTM) was similar to unheated SSBM. On the other hand, Lys concentration declined by 4% at 180°C (close to the processing temperature of HTM) and then continued to decline to 87% of the unheated SSBM at 200°C. Except for hydroxyproline, which decreased by 19%, the concentration of all other AA was unaltered by heating at 200°C. A much sharper 16% decline in Lys concentration of SSBM heated to 160°C for 30 min was reported by Hsu and Satter (1995). These authors, however, also observed a slight (5%) increase in plasma Lys concentration in heifers with increasing heating temperature (up to 153°C), which is indicative of increased rumen stability and postruminal availability of Lys from heated SSBM, in spite of its decreased concentration. Ipharraguerre et al. (2005) also reported lower Lys concentration in expeller SBM compared with SSBM, but greater duodenal flow of EAA, including Lys, with the nonmicrobial fraction of N when expeller SBM was included in the diet of lactating dairy cows.

The ESBM diets contained urea (to achieve a similar N concentration to the SSBM diet) and total N intake was greater than the SSBM diet, which explains the increased urinary urea-N excretion with LTM and HTM observed in the current experiment. The urinary urea excretion data are in agreement with the increased MUN and the trends for increased rumen ammonia and PUN concentrations with the ESBM diets. These results are not surprising as the effects of increased total protein (or RDP) intake on rumen ammonia, urinary N excretion, and MUN are well documented (Olmos Colmenero and Broderick, 2006). The lack of effect of ESBM on fecal N excretion is in agreement with the similar total-tract N digestibility between SSBM and the ESBM diets. The ESBM diets did contain higher concentrations of total fat, originating from the higher fat content of the ESBM vs. SSBM, but this did not appear to negatively affect ruminal fermentation or total-tract fiber digestibility. Although milk fat concentration was slightly (numerically) lower for the ESBM diets, rumen VFA concentrations and acetate:propionate ratio were similar among the diets and *trans*-10, *cis*-12 CLA, implicated in milk fat depression (Baumgard et al., 2000), was not detected in milk fat.

Fibrobacterales were increased by the HTM diet (and was numerically higher for LTM), compared with SSBM, in the current experiment. *Fibrobacter* spp. are predominant fibrolytic bacteria in the rumen (Stewart and Flint, 1989), and it is possible that their proportional increase with the ESBM diets was due to the urea inclusion and the numerically higher rumen ammonia concentration with these diets; the preference for ammonia N of ruminal fibrolytic bacteria has been documented (Russell et al., 1992). Ammonia concentration in ruminal fluid was relatively low in this experiment compared with average values reported in a meta-analysis (Broderick et al., 2010) or our own data with low-protein diets (Lee et al., 2015). Thus, it is plausible that rumen ammonia may have limited certain fibrolytic species in the current experiment, which might have been partially alleviated by the addition of urea to the ESBM diets. The effect of HTM on other bacteria, such as *Barnesiella* and the saccharolytic *Dorea*, is difficult to explain. Rumen archaea were not affected by treatment in the current experiment, although the effect of fat, specifically unsaturated FA, on rumen methanogens and enteric methane emission is often reported in the literature (Hristov et al., 2013b).

CONCLUSIONS

This experiment used SBM extruded at 2 extruder temperatures that were compared with SSBM as part of TMR for lactating dairy cows. Protein from ESBM had slower degradation rate and lower rumen degradability compared with SSBM. The experimental data conclusively showed that ESBM, perhaps being more palatable to the cows, increased DMI and consequently milk yield without affecting milk composition or feed efficiency. The ESBM diets, likely as a result of inclusion of NPN as urea, numerically increased rumen ammonia concentration, and increased urinary urea N excretion and MUN concentration. The ESBM diets tended to decrease most milk FA with chain length up to C17 and increased C18 FA in milk. Overall, data from this crossover experiment suggest that substituting SSBM with ESBM in the diet will have a positive effect on feed intake and milk yield in dairy cows.

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