



## Lactational performance, enteric gas emissions, and plasma amino acid profile of dairy cows fed diets with soybean or canola meals included on an equal protein basis

C. F. A. Lage,<sup>1,2</sup> S. E. Räisänen,<sup>1</sup> H. Stefenoni,<sup>1</sup> A. Melgar,<sup>1</sup> X. Chen,<sup>1,3</sup> J. Oh,<sup>4</sup> M. E. Fetter,<sup>1</sup> D. M. Kniffen,<sup>1</sup> R. A. Fabin,<sup>5</sup> and A. N. Hristov<sup>1\*</sup>

<sup>1</sup>Department of Animal Science, The Pennsylvania State University, University Park 16802

<sup>2</sup>Department of Animal Science, School of Veterinary Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil 31270-901

<sup>3</sup>Agri-Food and Biosciences Institute, Hillsborough, United Kingdom BT26 6DR

<sup>4</sup>Cargill Animal Nutrition, Seongnam, South Korea 13630

<sup>5</sup>Fabin Bros. Farms, Indiana, PA 15701

### ABSTRACT

This study investigated the effects of feeding solvent-extracted canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM) on an equivalent crude protein basis on performance, plasma AA profiles, enteric gas emissions, milk fatty acids, and nutrient digestibility in lactating dairy cows. Fifteen Holstein cows (95 ± 20 d in milk) were used in a replicated 3 × 3 Latin square design experiment with 3 periods of 28 d each. Treatments were 3 diets containing 17.1% CM, 14.2% ESBM, or 13.6% SSBM (dry matter basis). Vegetable oil was added (canola oil for CM or soybean oil for SSBM) to equalize the ether extract concentration of the diets. Rumen-protected Met was supplemented targeting digestible Met supply of 2.2% of metabolizable protein in all diets. Canola meal increased dry matter intake (DMI) by 5.9 and 8.9% in comparison with ESBM and SSBM, respectively. Milk urea nitrogen was lowest in CM, followed by SSBM, and was highest for ESBM. No differences were observed in feed efficiency, energy-corrected milk yield, and milk composition or component yields among treatments. Cows fed CM emitted less enteric CH<sub>4</sub> per kg of DMI compared with both ESBM and SSBM, but CH<sub>4</sub> emission intensity (CH<sub>4</sub> per kg of energy-corrected milk) was similar among treatments. In summary, replacement of ESBM or SSBM with CM, on an equal crude protein basis, in the diet of lactating dairy cows enhanced DMI, but yields of energy-corrected milk and milk components and feed efficiency were similar among treatments.

**Key words:** canola meal, extruded soybean meal, solvent-extracted soybean meal, dairy cattle

### INTRODUCTION

Solvent-extracted soybean meal (SSBM) is a by-product from the oil industry widely used as a protein supplement in farm animal diets. Over the past 40 yr, increased production of canola oil in North America led to an increased use of its byproduct, canola meal (CM), as a protein source in dairy cow rations. Comparative performance of dairy cows fed diets with SSBM or CM have been studied and 2 meta-analyses concluded that CM generally gives similar or greater DMI, milk yield (MY), and milk protein yield (Huhtanen et al., 2011; Martineau et al., 2013).

Due to different environmental conditions during growth and harvest, as well as variations in cultivars and meal processing, Canadian solvent-extracted CM can vary substantially in its nutritional composition. A survey that analyzed CM samples from 12 Canadian processing plants over a 4-yr period observed that CM RUP content ranged from 43 to 51% (Broderick et al., 2016), which is greater than the average RUP value for CM reported in the current NRC (2001) of 35.7% and is comparable to the RUP content of heat-treated or extruded soybean meal (ESBM; 49.5%; Harper et al., 2019).

Extruded soybean meal, which is a different product from expeller soybean meal or other commercially available heat-treated SBM products, is produced by a process that includes initial grinding, preheating, and pressing of the beans through a die (Björck and Asp, 1983). Previous work by Giallongo et al. (2015) demonstrated increased DMI and consequently MY in dairy cows fed diets in which SSBM was substituted by ESBM. However, we are not aware of any research comparing ESBM to CM. Comparing CM to ESBM, rather than SSBM, is appropriate because canola seeds, due to their high oil content, undergo an extrusion process before solvent extraction of the oil (Unger, 1990).

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\*Corresponding author: [anh13@psu.edu](mailto:anh13@psu.edu)

This extrusion, similar to the ESBM process, creates heat due to friction that increases RUP content of the resulting CM.

Therefore, we hypothesized that an ESBM-based diet would be equivalent to a CM-based diet in terms of lactational performance of dairy cows, but the CM diet may outperform a SSBM-based diet. The specific objectives of the study were to investigate the effects of these 3 feed protein sources, fed on an equal CP basis (with added rumen-protected Met and vegetable oil), on lactational performance, plasma AA profiles, enteric gas emissions, milk fatty acids (**FA**), digestibility, and N excretions in lactating dairy cows.

## MATERIALS AND METHODS

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

### *Animals and Experimental Design*

The experiment was conducted in the tiestall barn of The Pennsylvania State University's Dairy Teaching and Research Center. Fifteen lactating Holstein cows (3 primiparous and 12 second-lactation cows), averaging ( $\pm$ SD) 95 ( $\pm$ 20.0) DIM, 46.5 ( $\pm$ 7.80) kg/d MY, and 585 ( $\pm$ 37.8) kg of BW at the beginning of the study were used in a replicated 3  $\times$  3 Latin square design balanced for carryover effects. Cows were grouped into 5 squares based on parity, DIM, and MY. Each experimental period lasted 28 d, with the first 18 d for adaptation to the diets, followed by 10 d for data and sample collection. Cows within square were randomly assigned to 1 of 3 treatment diets fed as TMR containing CM, ESBM, or SSBM as the main source of feed protein, included at 17.1%, 14.2%, and 13.6% of DM, respectively (Table 1). Our goal was to feed the same amount of CP from each protein source, rather than have the same physical inclusion rate. Vegetable oil was added to the SSBM (soybean oil; Sam's Club, Bentonville, AR) and CM (canola oil; Sam's Club, Bentonville, AR) diets to match the ether extract concentration of the ESBM diet. Rumen-protected Met (Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) was used to achieve a target digestible Met supply of 2.2% of MP in all diets. Rumen escape and intestinal digestibility of Met in Mepron were assumed to be 80 and 90%, respectively, according to the manufacturer's specification. The Met concentration of diets was calculated based on AA analysis of CM, ESBM, and SSBM (analytical procedures specified below) and AA values based on NRC (2001) library for all other

feed ingredients. Rumen-degraded and RUP values for the protein meals were derived from the in situ experiment (see below) and NRC (2001) feed library data were used for all other feed ingredients.

All 3 diets had similar ingredient composition and were formulated to meet or exceed  $NE_L$  and MP requirements (NRC, 2001) of a Holstein cow producing 46 kg of milk/d with 3.30% milk fat and 2.85% true protein at 27 kg/d DMI and BW of 611 kg. The SSBM was locally sourced (Cargill Inc., Roaring Spring, PA) and the ESBM was produced by Fabin Bros. (Indiana, PA) at 171°C extrusion temperature. The CM was purchased through Gavilon Group LLC (Omaha, NE) and was sourced from processing plants in Canada (Bunge in Harrowby, MB, and ADM in Windsor, ON, Canada). The 3 diets were mixed using a mobile mixer (Rissler Mobile TMR Mixer Model 1050, I. H. Rissler, Mohnton, PA) and were fed once daily (0700 h) as TMR to achieve 10% refusals. All cows had free access to drinking water and were milked twice daily (at approximately 0600 and 1800 h).

### *Sampling and Measurements*

Individual feed intake (on as-fed basis) and MY of the cows were recorded daily throughout the experiment. Cow BW was also recorded twice daily for the entire experiment using an 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 the straw-hay mix) 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 feed samples were stored at  $-20^\circ\text{C}$  until analysis. Samples were dried for DM determination at  $55^\circ\text{C}$  for 72 h in a forced-air oven, and ground in a Wiley Mill (1-mm screen; Thomas Scientific, Swedesboro, NJ) for further analyses. Feed DMI was calculated 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 (method 990.03; AOAC International, 2000), RDP and RUP (CM, ESBM, and SSBM only; using an in situ procedure described below), amylase-treated NDF (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), ether extract (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), Ca and P (method 985.01;

**Table 1.** Ingredients and chemical composition of diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM) fed in the experiment

Item	Diet		
	CM	ESBM	SSBM
Ingredient, % of DM <sup>1</sup>			
Corn silage <sup>2</sup>	45.1	45.1	45.2
Alfalfa haylage <sup>3</sup>	11.0	11.0	11.0
Hay-straw mixture <sup>4</sup>	1.99	5.49	5.21
Whole cottonseed	5.98	5.99	5.98
Corn grain, ground	9.90	9.90	9.89
SSBM	—	—	13.6
CM	17.1	—	—
ESBM	—	14.2	—
SoyPLUS <sup>5</sup>	1.99	1.99	1.99
Molasses <sup>6</sup>	4.91	4.91	4.91
Vegetable oil <sup>7</sup>	0.66	—	0.89
Mepron <sup>8</sup>	0.05	0.08	0.08
Mineral and vitamin premix <sup>9</sup>	1.29	1.29	1.29
CP supply from protein meals, <sup>10</sup> g/kg of DMI	70.0	70.0	69.0
Composition, % of DM			
CP <sup>11</sup>	16.3	16.7	16.6
RDP <sup>12</sup>	8.20	8.70	9.50
RUP <sup>12</sup>	8.10	8.00	7.10
NDF <sup>11</sup>	32.9	31.4	30.9
ADF <sup>11</sup>	22.2	20.7	20.6
Ether extract <sup>11</sup>	5.00	5.10	5.00
NE <sub>L</sub> , <sup>11</sup> Mcal/kg of DM	1.54	1.58	1.58
NE <sub>L</sub> balance, <sup>12</sup> Mcal/d	0.60	-0.10	-0.20
MP balance, <sup>12,13</sup> g/d	93.0	288	81.0
Digestible Met, <sup>12,13</sup> g/d	60.0	61.0	59.0
NFC <sup>12</sup>	42.9	43.3	43.8
Ca <sup>11</sup>	0.72	0.64	0.69
P <sup>11</sup>	0.44	0.36	0.35

<sup>1</sup>Or as indicated.<sup>2</sup>Corn silage was 44.4% DM and contained (DM basis) 7.3% CP and 39.0% NDF.<sup>3</sup>Alfalfa haylage was 31.9% DM and contained (DM basis) 21.7% CP and 41.3% NDF.<sup>4</sup>Chopped (Standard Roto Grind Model 760, Roto Grind Tub Grinders, Greeley, CO) hay-straw mixture contained (DM basis) 11.5% CP.<sup>5</sup>SoyPLUS (West Central Cooperative, Ralston, IA) contained (DM basis) 46.6% CP.<sup>6</sup>Liquid molasses was from Westway Feed Products (Tomball, TX).<sup>7</sup>Vegetable oil: canola oil in CM diet and soybean oil in SSBM diet.<sup>8</sup>Mepron is a rumen-protected Met source (Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany): 80% rumen by-pass fraction and 90% intestinal digestibility, based on manufacturer's specifications.<sup>9</sup>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.<sup>10</sup>Crude protein supply from CM, ESBM, and SSBM, respectively.<sup>11</sup>Values calculated using the chemical analysis (Cumberland Valley Analytical Services Inc., Waynesboro, PA) of the feed ingredients and their inclusion in the diets.<sup>12</sup>Estimated based on NRC (2001) and in situ data from the current experiment for the protein meals using actual DMI, milk yield, milk composition, and BW of the cows throughout the experiment.<sup>13</sup>Metabolizable protein balance estimated based on NRC (2001) using CM, ESBM, and SSBM protein degradability values estimated in the current in situ experiment.

AOAC International, 2000), and estimated NFC and NE<sub>L</sub> by Cumberland Valley Analytical Services Inc. (Waynesboro, PA). The analyzed composition of the feed ingredients and their inclusion rate in the TMR were used to compute CP, NDF, ADF, ether extract, Ca, and P concentration of the diets (Table 1). Dietary supplies of NE<sub>L</sub>, MP, and digestible Met were estimated using NRC (2001) based on actual DMI, MY, milk composition, and BW of the cows during the experiment. Concentrations of MP, RDP, and RUP were estimated based on in situ data derived in the current study for CM, ESBM, and SSBM and NRC (2001) values for the other feed ingredients. Composite TMR samples were analyzed for starch according to Hall (2009) and indigestible NDF (iNDF) as described by Huhtanen et al. (1994) and modified by Lee et al. (2012). Samples of SSBM, CM, and ESBM were also analyzed for AA composition at the University of Missouri-Columbia's Agricultural Experiment Station Chemical Laboratory (Columbia, MO) following the procedures of Deyl et al. (1986) and Fekkes (1996). Amino acid composition of the other feed ingredients was analyzed with ion-exchange chromatography by Evonik Nutrition & Care GmbH (Hanau-Wolfgang, Germany; AOAC International, 1995; European Commission, 2009). Intestinal digestibility of CM, ESBM, and SSBM protein was analyzed at Rock River Laboratories Inc. (Watertown, WI) using the 3-step procedure of Calsamiglia and Stern (1995).

During the last week of each experimental period, 8 spot fecal and urine samples were collected in 3 consecutive days at intervals staggered in time to cover a 24-h period starting at 0500, 1100, 1700, and 2300 h (d 1), 0800, 1400, and 2000 h (d 2), and 0200 h (d 3). Fecal samples were oven-dried at 55°C for 72 h, ground through a 1-mm sieve in a Wiley Mill (Thomas Scientific), composited per cow and experimental period, and then analyzed DM, OM, CP, NDF, ADF, iNDF, and starch as described above. Total-tract apparent digestibility of DM, OM, NDF, ADF, CP, and starch was estimated using iNDF as an internal digestibility marker (Schneider and Flatt, 1975). Urine samples were processed and analyzed for allantoin, uric acid, creatinine, urinary urea N (UUN), and total N. Total N was analyzed in freeze-dried urine samples of approximately 60 µL of 1:10 diluted and acidified urine using a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). Stanbio Laboratory (Boerne, TX) kits were used to analyze UUN (Urea Nitrogen Kit 580), creatinine (Creatinine Kit 420), and uric acid (Uric Acid Kit 1045). Allantoin was analyzed following the procedure by Chen et al. (1992). Daily volume of excreted urine was estimated based on urinary creatinine concentration,

assuming a creatinine excretion rate of 29 mg/kg of BW based on total urine collection data from Hristov et al. (2011b). Daily total N, urinary urea, and purine derivative excretions were calculated based on the estimated urine output.

Blood samples were collected from the tail vein or artery into EDTA vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) 4 times in 2 consecutive days at 0900 and 1700 h (d 1) and 1400 and 2000 h (d 2). Blood plasma was separated, processed (Lee et al., 2012), and composited on an equal volume basis per cow and period for analysis of AA at the University of Missouri–Columbia’s Agricultural Experiment Station Chemical Laboratory as described above.

Milk samples were collected from 2 consecutive milkings (evening and morning) on 2 separate days (i.e., a total of 4 milkings) during wk 4 of each experimental period. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol and submitted to Dairy One Laboratory (Ithaca, NY) for analysis of fat, true protein, lactose, and MUN using infrared spectroscopy and Milkoscan models 6000, FT+, or 7 and Fossomatic models 5000 of FC (Foss Electric A/S, Hillerød, Denmark). Energy-corrected milk was calculated according to Sjaunja et al. (1990):  $ECM \text{ (kg/d)} = \text{kg of milk} \times [(38.3 \times \% \text{ fat} \times 10 + 24.2 \times \% \text{ true protein} \times 10 + 16.54 \times \% \text{ lactose} \times 10 + 20.7) \div 3,140]$ . Evening and morning milk samples were analyzed separately to weigh the milk component concentrations for evening and morning MY. A separate unpreserved milk sample (from all 4 milkings) was stored at  $-20^{\circ}\text{C}$ , composited on an equal volume basis per cow and period, and analyzed for FA as described by Rico and Harvatine (2013).

Enteric  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{H}_2$  emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD) as described by Hristov et al. (2015). Briefly, measurements occurred 8 times in 3 d, covering a 24-h period as follows: 0900, 1500, and 2100 h (d 1), 0300, 1200, and 1700 h (d 2), and 0000 and 0500 h (d 3). Individual breath gas samples were collected for 5 min, followed by 2-min background air sample collection.

A subexperiment was conducted to determine in situ ruminal degradability of CP of CM, ESBM, and SSBM. Three cows were used in the in situ experiment and were fed (% of DM) the following: corn silage, 43.4; alfalfa haylage, 12.0; grass-hay mix, 3.40; ground corn grain, 8.70; whole roasted soybeans, 8.00; SoyPLUS (Landus Cooperative, Ames, IA), 5.01; canola meal, 8.49; cottonseed hulls, 5.01; molasses, 4.49; and a mineral and vitamin premix, 1.50 (Cargill Animal Nutrition, Cargill Inc., Roaring Spring, PA). The experimental procedures were as described by Lee et al. (2012). Samples

of all 3 meals were collected throughout the experiment and composited at the end of the experiment. The composite samples were used for the in situ experiment. Triplicate samples of 7 g each (as-fed basis and without further processing) were weighed into Ankom nylon bags (10 cm  $\times$  20 cm forage bag; Ankom Technology Corp., Macedon, NY), which were sequentially incubated in the ventral rumen for 2, 4, 8, 24, and 48 h and simultaneously removed. It is noted that a 16-h time point (recommended by NRC, 2001) was not used in the in situ experiment. Samples were not pre-soaked before placed into the rumen. Following incubation, all bags were manually washed, including the 0 h samples, which were not incubated in the rumen, with cold tap water until the water ran clean. All samples were oven-dried for 72 h at  $55^{\circ}\text{C}$  and aliquots of each bag residue were pulverized using a Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) and analyzed for N using an elemental analyzer (Costech ECS 4010 C/N/S, Costech Analytical Technologies Inc., Valencia, CA) to calculate CP (N  $\times$  6.25). Ruminal disappearance was calculated based on average per cow and incubation time point, of initial dry weight of the incubated sample, dry weight of the residues, and N content of the incubated samples and bag residues. Degradation data were fitted to a 3-parameter, exponential rise to a maximum model:  $p = a + b \times (1 - e^{-ct})$ , with the constraint that  $a + b \leq 100\%$ , using SigmaPlot v. 10.0 (Systat Software Inc., San Jose, CA), where  $p$  is the degraded fraction of CP at time  $t$ ,  $a$  is the soluble fraction (or intercept),  $b$  is the potentially degradable fraction of CP, and  $c$  is the rate of degradation of fraction  $b$  (Ørskov and McDonald, 1979). Effective degradability (**ED**; percentage of CP that would be potentially degraded in the rumen at specified passage rate) was estimated using the equation of Ørskov and McDonald (1979):  $ED = a + b \times [c \div (c + k)]$ , where  $a$ ,  $b$ , and  $c$  are as specified above and  $k$  is the rate of passage assumed to be 6%/h.

### Statistical Analysis

All data were analyzed using the MIXED procedure of SAS, version 9.4 (SAS Institute Inc., Cary, NC). Milk yield and DMI data for the last 10 d of each experimental period were used in the statistical analysis. Feed efficiency (MY  $\div$  DMI) was estimated based on MY and DMI data over the last 10 d of each experimental period. These data were analyzed as repeated measures. The statistical model included treatment, experimental period, the repeated term (day), and treatment  $\times$  day interaction. Square and cow within square were random effects and all others were fixed. The best covariance structure for repeated measures was chosen by the low-

est corrected Akaike information criterion, which was AR(1) for DMI and MY, VC for BW, and CS for feed efficiency. Milk composition and component yield data were averaged per cow and per period, and the average values were used in the statistical analysis and to calculate ECM. For ECM feed efficiency ( $ECM \div DMI$ ), the 10-d average DMI was used. Enteric gas emission data were averaged across all sampling points and the average values per cow and per period were used in the statistical analysis. Milk composition, plasma AA, milk FA, enteric gas emissions, digestibility, and N and purine derivative excretion data were analyzed with the model described above, excluding the repeated term and its interaction with treatment. The in situ degradability data were analyzed with protein source in the model. When the main effect of treatment or protein source were significant, means were separated by pairwise *t*-test (pdiff option of PROC MIXED) with Tukey's adjustment. Mean differences were considered significant at  $P \leq 0.05$ , trends were declared at  $0.05 < P \leq 0.10$ , and numerical differences were declared at  $0.10 < P \leq 0.15$ . Data are presented as least squares means.

## RESULTS

### Diet and Feed Composition

Dietary ingredients and chemical composition of the diets fed are shown in Table 1. All 3 diets had similar ingredient composition, except that the SBM diets had a greater hay-straw mixture inclusion to achieve similar NDF values as for the CM diet. Differences in the CP content of the hay-straw mixture throughout the experiment resulted in slight differences in CP concentration of the CM and SBM diets.

The CM diet provided  $NE_L$  in excess of the cow's requirements (NRC, 2001) and the ESBM and SSBM diets were slightly below  $NE_L$  requirements. All 3 diets provided MP and RUP in excess of the NRC (2001) recommendations, but were 15.7, 11.6, and 3.8% deficient in estimated RDP supply (CM, ESBM, and SSBM diets, respectively; RDP and RUP balance not shown in Table 1). In addition, diets provided an estimated (NRC, 2001) digestible Met supply of 1.96, 1.93, and 2.05% of MP (respectively), which was below the 2.2% recommended by Schwab et al. (2005).

Chemical and AA composition of the meals is presented in Table 2. The ESBM and SSBM had 20 and 25% (respectively) greater CP concentration than CM. The RUP content of CM was greater than expected based on average NRC (2001) values (36% of CP). Likewise, analyzed RUP content of SSBM was 14.4%

greater than the average NRC (2001) value (43%). Intestinal digestibility of RUP was greater for ESBM, in comparison with CM and SSBM (by 21.6% and 7.8%, respectively). As expected, CM had on average 3.8 times greater NDF content than the SBM meals.

Concentrations of Arg, Ile, Leu, Lys, Phe, and Trp in the meal CP were greater in SBM than CM. Concentrations of Thr, Val, and Met were greater in CM than SBM. Overall, concentration of total EAA was greater for the SBM products than CM. Concentrations of most individual and total NEAA were greater in SBM than CM, except for Cys, Gly, and Pro, which

**Table 2.** Chemical composition and AA concentration of canola meal (CM), extruded soybean meal (ESBM), and solvent-extracted soybean meal (SSBM) fed to lactating dairy cows in the experiment

Item	Protein meal		
	CM	ESBM	SSBM
CP, <sup>1</sup> % DM	40.7	49.0	51.0
RDP, <sup>2</sup> % of CP	36.8	38.9	50.8
RUP, <sup>2</sup> % of CP	63.2	61.1	49.2
RUP intestinal digestibility, <sup>3</sup> % of RUP	81.8	99.5	92.3
NDF <sup>1</sup>	30.3	9.00	6.90
ADF <sup>1</sup>	20.7	3.90	4.30
Ash <sup>1</sup>	7.73	5.99	7.74
Ca <sup>1</sup>	0.86	0.29	0.75
P <sup>1</sup>	1.16	0.72	0.70
EAA, <sup>4</sup> % of CP			
Arg	5.58	7.35	7.27
His	2.68	2.65	2.58
Ile	4.28	5.05	4.92
Leu	6.93	7.79	7.61
Lys	5.85	6.68	6.41
Met	1.99	1.39	1.37
Phe	3.92	5.16	5.02
Thr	4.14	3.85	3.72
Trp	1.16	1.50	1.34
Val	5.05	4.92	4.77
Total EAA	41.6	46.3	45.0
NEAA, <sup>4</sup> % of CP			
Ala	4.36	4.42	4.29
Asp	5.58	7.35	7.27
Cys	2.57	1.50	1.47
Glu	16.5	18.3	17.8
Gly	5.13	4.44	4.37
Pro	6.40	5.50	5.40
Ser	3.42	4.31	4.16
Tyr	2.54	3.63	3.61
Total NEAA	46.5	49.4	48.3
Total EAA and NEAA	88.1	95.7	93.3

<sup>1</sup>Analyzed by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) using wet chemistry methods.

<sup>2</sup>Estimated using in situ values and NRC (2001) equations to calculate RDP and RUP.

<sup>3</sup>Analyzed by Rock River Laboratories Inc. (Watertown, WI) using the Calsamiglia and Stern (1995) method.

<sup>4</sup>Analyzed by University of Missouri-Columbia's Agricultural Experiment Station Chemical Laboratories (Columbia, MO) following the procedures of Deyl et al. (1986) and Fekkes (1996).

**Table 3.** Ruminal in situ degradability of canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM) CP<sup>1</sup>

Item	Meal			SEM	P-value
	CM	ESBM	SSBM		
Soluble fraction ( <i>a</i> ), %	5.80	10.8	13.1	1.52	0.10
Potentially degradable fraction ( <i>b</i> ), %	94.2	89.2	86.9	1.51	0.10
Rate of degradation of <i>b</i> , %/h	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.05 <sup>a</sup>	0.004	0.01
Effective degradability, <sup>2</sup> %	36.7 <sup>b</sup>	38.8 <sup>b</sup>	50.6 <sup>a</sup>	2.06	0.01

<sup>a,b</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Values are model estimates of in situ CP disappearance curves. Data were fitted to the equation  $a + b \times (1 - e^{-ct})$ , with the constraint that  $a + b \leq 100\%$ , using SigmaPlot v. 10.0 (Systat Software Inc., San Jose, CA), where  $p$  is the degraded fraction of CP at time  $t$ ,  $a$  is the soluble fraction of CP (or intercept),  $b$  is the potentially degradable fraction of CP, and  $c$  is the rate of degradation of fraction  $b$  (Ørskov and McDonald, 1979).

<sup>2</sup>Effective degradability (ED) was estimated using the equation of Ørskov and McDonald (1979):  $ED = a + b \times [c \div (c + k)]$ , where  $a$ ,  $b$ , and  $c$  are as specified above and  $k$  is the rate of passage assumed to be 6%/h.

were greater in CM than in SBM. The sum of EAA + NEAA was on average 7% lower for CM in comparison with ESBM and SSBM.

The in situ CP degradability data for the 3 meals are shown in Table 3. The soluble fraction ( $a$ ) tended to be greater ( $P = 0.10$ ) and the potentially degradable fraction ( $b$ ) of CP tended to be lower ( $P = 0.10$ ) for SBM than CM. The rate of degradation of fraction  $b$  CP was 67% greater ( $P = 0.01$ ) for SSBM than CM or ESBM. Effective degradability of CP, estimated at an assumed

6%/h rate of passage, was also 38 and 30% greater ( $P = 0.01$ ) for SSBM than CM and ESBM, respectively.

### Feed Intake and Milk Production and Composition

The CM diet increased ( $P < 0.001$ ) DMI by 2.4 and 1.6 kg/d compared with ESBM and SSBM diets, respectively (Table 4). Compared with SSBM, the CM and ESBM diets increased ( $P = 0.002$ ) MY by 2.7 and 1.5 kg/d, respectively. No differences in MY between

**Table 4.** Dry matter intake, BW, and milk production variables in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
DMI, kg/d	26.9 <sup>a</sup>	25.3 <sup>b</sup>	24.5 <sup>b</sup>	0.82	<0.001 <sup>3</sup>
Milk yield, kg/d	43.8 <sup>a</sup>	42.6 <sup>a</sup>	41.1 <sup>b</sup>	1.89	0.002
Milk yield ÷ DMI, kg/kg	1.64	1.70	1.70	0.039	0.35
Milk fat, %	3.66	3.64	3.65	0.125	0.99
Milk fat, kg/d	1.60	1.55	1.54	0.092	0.67
Milk true protein, %	3.10	3.09	3.17	0.041	0.17
Milk true protein, kg/d	1.36	1.32	1.34	0.077	0.82
Milk lactose, %	4.78	4.85	4.81	0.036	0.19
Milk lactose, kg/d	2.10	2.08	2.05	0.105	0.86
MUN, mg/dL	9.23 <sup>c</sup>	12.0 <sup>a</sup>	10.4 <sup>b</sup>	0.40	<0.001
ECM, <sup>4</sup> kg/d	41.4	40.3	40.3	2.20	0.74
ECM ÷ DMI, kg/kg	1.56	1.65	1.66	0.063	0.32
Milk NE <sub>L</sub> , <sup>5</sup> Mcal/d	30.8	30.1	30.0	1.64	0.74
BW, kg	602	598	594	5.79	0.11

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table;  $n = 438$  for DMI;  $n = 421$  for milk yield;  $n = 413$  for milk yield ÷ DMI;  $n = 44$  for milk composition variables; and  $n = 450$  for BW ( $n$  represents number of observations used in the statistical analysis). Data are presented as LSM.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>ESBM versus SSBM,  $P = 0.12$ .

<sup>4</sup>Sjaunja et al. (1990).

<sup>5</sup>According to NRC (2001).

CM and ESBM diets were observed and treatments did not affect feed efficiency. Milk urea nitrogen concentration was lowest ( $P < 0.001$ ) in CM, followed by SSBM, and was highest for ESBM. Treatments did not affect milk composition and component yields, ECM yield, and ECM feed efficiency. There was a trend for lower ( $P = 0.11$ ) BW of the cows when fed the CM versus both SBM diets, but this experiment was not designed to investigate effects on BW.

### Plasma Amino Acids

Compared with CM and SSBM, the ESBM diet increased ( $P \leq 0.05$ ) plasma concentrations of Ile, Leu,

Val, and the sum of EAA and had lower ( $P < 0.005$ ) Met concentration (Table 5). In addition, the ESBM diet increased ( $P < 0.005$ ) plasma concentrations of Cit and decreased that of Cys compared with the CM and SSBM diets. The SSBM diet had lower concentration of 1-methylhistidine than both ESBM and CM ( $P < 0.001$ ). The CM diet had lower ( $P < 0.001$ ) plasma urea concentration in comparison with the SBM diets.

### Milk Fatty Acids

Compared with ESBM and SSBM, the CM diet increased ( $P \leq 0.03$ ) concentrations of 18:1, 20:0, and MUFA in milk fat (Table 6). On the other hand, ESBM

**Table 5.** Blood plasma AA concentration ( $\mu\text{M}$ ) in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
Arg	78.9	84.0	79.1	4.56	0.29
His	48.0	51.4	46.3	2.45	0.29
Ile	129 <sup>b</sup>	153 <sup>a</sup>	128 <sup>b</sup>	5.52	<0.001
Leu	142 <sup>b</sup>	171 <sup>a</sup>	132 <sup>b</sup>	5.75	<0.001
Lys	80.6	83.2	80.1	3.33	0.69
Met	25.4 <sup>a</sup>	20.0 <sup>b</sup>	23.0 <sup>a</sup>	0.92	0.001
Phe	46.4 <sup>ab</sup>	49.1 <sup>a</sup>	42.2 <sup>b</sup>	1.59	0.005
Thr	102	93.4	95.6	5.27	0.36
Trp	30.0 <sup>a</sup>	29.4 <sup>ab</sup>	27.3 <sup>b</sup>	0.93	0.04
Val	271 <sup>b</sup>	302 <sup>a</sup>	250 <sup>b</sup>	9.15	<0.001
$\Sigma\text{EAA}^3$	953 <sup>b</sup>	1,036 <sup>a</sup>	904 <sup>b</sup>	30.0	0.002
$\Sigma\text{EAA without Met}$	927 <sup>b</sup>	1,016 <sup>a</sup>	881 <sup>b</sup>	29.5	0.001
Ala	263	236	245	10.2	0.08
Asn	44.5	50.4	46.0	2.29	0.07
Asp	5.09 <sup>b</sup>	7.09 <sup>a</sup>	6.23 <sup>ab</sup>	0.58	0.02
Cit	71.2 <sup>b</sup>	80.6 <sup>a</sup>	70.9 <sup>b</sup>	3.45	0.006
Cys	1.95 <sup>a</sup>	1.27 <sup>c</sup>	1.59 <sup>b</sup>	0.08	<0.001
Gln	243	241	249	9.54	0.66
Glu	54.6	53.8	51.9	2.16	0.50
Gly	283	279	286	11.8	0.87
Orn	43.8	48.4	42.4	2.24	0.10
Pro	78.7	82.3	74.5	4.10	0.16
Ser	76.5	79.8	74.3	3.41	0.24
Tau	50.2	50.4	47.5	3.80	0.64
Tyr	48.1	48.6	44.4	1.93	0.10
$\Sigma\text{NEAA}^4$	1,273	1,268	1,251	35.0	0.85
$\Sigma\text{TAA}^5$	2,226	2,304	2,154	60.0	0.11
Carnosine	14.2	14.3	13.5	0.55	0.39
1-MH <sup>6</sup>	18.0 <sup>a</sup>	17.7 <sup>a</sup>	15.0 <sup>b</sup>	1.43	<0.001
3-MH <sup>6</sup>	3.77	3.57	3.47	0.17	0.22
Urea	3,433 <sup>b</sup>	4,695 <sup>a</sup>	4,397 <sup>a</sup>	114	<0.001

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table; n = 45 (n represents number of observations used in the statistical analysis).

<sup>2</sup>Main effect of treatment.

<sup>3</sup>Sum of EAA (Arg, His, Ile, Leu, Lys, Phe, Thr, Trp, and Val).

<sup>4</sup>Sum of NEAA (Ala, Asn, Asp, Cys, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tau, and Tyr were considered as NEAA).

<sup>5</sup>Sum of total AA.

<sup>6</sup>MH = methylhistidine.

**Table 6.** Fatty acid (FA) composition of milk fat (g/100 g of total FA) in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
4:0	4.52	4.65	4.41	0.11	0.07
6:0	2.32 <sup>b</sup>	2.43 <sup>a</sup>	2.35 <sup>ab</sup>	0.04	0.02
8:0	1.27 <sup>b</sup>	1.34 <sup>a</sup>	1.30 <sup>ab</sup>	0.03	0.02
10:0	2.82	2.92	2.96	0.11	0.13
12:0	3.15	3.23	3.37	0.13	0.11
14:0	10.1 <sup>b</sup>	10.3 <sup>ab</sup>	10.7 <sup>a</sup>	0.24	0.02
<i>cis</i> -9 14:1	0.81 <sup>b</sup>	0.87 <sup>ab</sup>	0.92 <sup>a</sup>	0.03	0.03
15:0	1.02 <sup>ab</sup>	0.94 <sup>b</sup>	1.10 <sup>a</sup>	0.049	0.04
16:0	26.1 <sup>b</sup>	27.0 <sup>b</sup>	28.6 <sup>a</sup>	0.872	<0.001
<i>cis</i> -9 16:1	1.25 <sup>b</sup>	1.26 <sup>ab</sup>	1.37 <sup>a</sup>	0.089	0.02
17:0	0.51 <sup>a</sup>	0.48 <sup>b</sup>	0.51 <sup>a</sup>	0.011	0.003
18:0	12.1 <sup>a</sup>	11.1 <sup>ab</sup>	10.5 <sup>b</sup>	0.479	0.004
<i>trans</i> -4 18:1	0.04 <sup>a</sup>	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.002	<0.001
<i>trans</i> -5 18:1	0.023 <sup>a</sup>	0.017 <sup>b</sup>	0.016 <sup>b</sup>	0.001	<0.001
<i>trans</i> -6,8 18:1	0.44 <sup>a</sup>	0.36 <sup>b</sup>	0.36 <sup>b</sup>	0.018	<0.001
<i>trans</i> -9 18:1	0.35 <sup>a</sup>	0.30 <sup>b</sup>	0.30 <sup>b</sup>	0.011	<0.001
<i>trans</i> -10 18:1	0.77	0.72	0.71	0.060	0.62
<i>trans</i> -11 18:1	1.39	1.33	1.31	0.089	0.52
<i>trans</i> -12 18:1	0.65 <sup>a</sup>	0.59 <sup>b</sup>	0.61 <sup>ab</sup>	0.031	0.05
<i>cis</i> -9 18:1	19.8 <sup>a</sup>	18.8 <sup>b</sup>	18.1 <sup>b</sup>	0.469	0.001
<i>cis</i> -11 18:1	1.05 <sup>a</sup>	0.78 <sup>b</sup>	0.78 <sup>b</sup>	0.029	<0.001
<i>cis</i> -9, <i>cis</i> -12 18:2	2.22 <sup>b</sup>	3.18 <sup>a</sup>	2.26 <sup>b</sup>	0.078	<0.001
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.018 <sup>c</sup>	0.028 <sup>a</sup>	0.023 <sup>b</sup>	0.001	<0.001
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.40 <sup>b</sup>	0.50 <sup>a</sup>	0.36 <sup>c</sup>	0.010	<0.001
20:0	0.14 <sup>a</sup>	0.12 <sup>b</sup>	0.11 <sup>b</sup>	0.004	<0.001
<i>cis</i> -9, <i>trans</i> -11 CLA	0.59	0.61	0.62	0.037	0.51
Others	2.34	2.24	2.37	0.094	0.12
Total <i>trans</i> FA	4.17	3.85	3.83	0.198	0.05
ΣSFA	66.0 <sup>b</sup>	66.5 <sup>b</sup>	68.0 <sup>a</sup>	0.631	0.002
ΣMUFA	28.1 <sup>a</sup>	26.6 <sup>b</sup>	26.1 <sup>b</sup>	0.541	<0.001
ΣPUFA	3.50 <sup>b</sup>	4.66 <sup>a</sup>	3.57 <sup>b</sup>	0.109	<0.001
ΣDe novo <sup>3</sup>	25.2 <sup>b</sup>	26.0 <sup>ab</sup>	26.3 <sup>a</sup>	0.43	0.03
ΣMixed	27.4 <sup>b</sup>	28.3 <sup>b</sup>	30.0 <sup>a</sup>	0.929	<0.001
ΣPreformed	41.5 <sup>a</sup>	40.0 <sup>a</sup>	37.6 <sup>b</sup>	0.898	<0.001
ΣOBCFA <sup>4</sup>	3.62 <sup>ab</sup>	3.43 <sup>b</sup>	3.78 <sup>a</sup>	0.076	0.001

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table; n = 45 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>De novo FA (<C16) are synthesized by the mammary gland; preformed FA (>C16) originate primarily from extraction from plasma; and mixed FA (C16) originate from both sources.

<sup>4</sup>Odd- and branched-chain FA. Sum of C11:0, *iso* C13:0, *anteiso* C13:0, C13:0, *iso* C15:0, *anteiso* C15:0, C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, and C17:1 *cis*-9.

increased ( $P < 0.001$ ) concentrations of 17:0, 18:2, 18:3, and PUFA in comparison with both CM and SSBM. The SSBM diet increased ( $P < 0.002$ ) concentrations of 16:0 and SFA and decreased ( $P < 0.001$ ) preformed FA.

### Enteric Gas Emissions

Daily enteric CH<sub>4</sub> emission was similar among diets, but cows fed CM had lower ( $P = 0.006$ ) CH<sub>4</sub> yield (i.e., CH<sub>4</sub>/kg DMI) than cows fed the ESBM and SSBM diets (Table 7). The CM diet had lower ( $P = 0.004$ ) CH<sub>4</sub> g/kg of digested OM in comparison with the SSBM diet. Methane emission intensity (i.e., CH<sub>4</sub>/kg of ECM

milk) was similar among treatments. Hydrogen and CO<sub>2</sub> emissions were not different among treatments.

### Apparent Total-Tract Digestibility and Nitrogen Excretion

Intake of all nutrients during the digestibility measurement periods was greater ( $P < 0.004$ ) for CM compared with ESBM and SSBM diets (Table 8). Apparent total-tract digestibility of DM, OM, and starch was lower ( $P < 0.02$ ) for SSBM in comparison with ESBM, but no differences between CM and SSBM diets were observed. The SSBM diet also had lower ( $P = 0.002$ )



**Table 7.** Enteric gas emissions in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
CH <sub>4</sub> , g/d	396	411	414	17.2	0.46
CH <sub>4</sub> , g/kg of DMI	15.0 <sup>b</sup>	16.9 <sup>a</sup>	17.0 <sup>a</sup>	0.85	0.006
CH <sub>4</sub> , g/kg of digested OM	23.6 <sup>b</sup>	25.8 <sup>ab</sup>	27.0 <sup>a</sup>	1.31	0.004
CH <sub>4</sub> <sup>3</sup> , g/kg of ECM	9.53	9.94	10.4	0.60	0.18
CO <sub>2</sub> , g/d	13,118	12,868	13,163	339	0.38
H <sub>2</sub> , g/d	0.49	0.43	0.48	0.05	0.27

<sup>a,b</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table;  $n = 45$  for all variables except CH<sub>4</sub>, g/kg of ECM,  $n = 44$  ( $n$  represents the number of observations used in the statistical analysis). Data are presented as LSM.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>Sjaunja et al. (1990).

NDF digestibility than both CM and ESBM diets. The ESBM diet had the greatest ( $P < 0.001$ ) CP digestibility and there was no difference in CP digestibility between CM and SSBM.

Nitrogen intake was greater ( $P = 0.002$ ) for CM in comparison with SSBM and ESBM diets (Table 9). Daily fecal N excretion was greater ( $P < 0.001$ ) for CM compared with the ESBM diet and tended ( $P = 0.09$ ) to be greater for CM than the SSBM diet. However, fecal N excretion as % of N intake was similar between CM and SSBM and was lower ( $P = 0.001$ ) for the ESBM diet. Diet had no effect on urinary N excretion, total excreta N, and milk N secretion; diets also had no effect on urinary excretion of purine derivatives. The ESBM diet had greater ( $P < 0.001$ ) daily UUN excretion

in comparison with both CM and SSBM, but UUN excretion as % of N intake was not different between ESBM and SSBM. Urinary creatinine concentration was greater ( $P = 0.003$ ) and consequently estimated urine output was lower ( $P = 0.014$ ) for CM, compared with the SBM diets.

## DISCUSSION

### Diet and Feed Composition

The diets used in this study were formulated based on NRC (2001). The discrepancy between analyzed RDP and RUP of CM (this study and Maxin et al., 2013b) and book values (i.e., NRC, 2001) suggests that

**Table 8.** Nutrient intake and apparent total-tract digestibility in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
Intake, <sup>3</sup> kg/d					
DM	26.8 <sup>a</sup>	24.6 <sup>b</sup>	24.3 <sup>b</sup>	0.84	<0.001
OM	25.2 <sup>a</sup>	23.1 <sup>b</sup>	22.9 <sup>b</sup>	0.79	<0.001
NDF	9.37 <sup>a</sup>	8.12 <sup>b</sup>	8.15 <sup>b</sup>	0.290	<0.001
ADF	5.52 <sup>a</sup>	4.60 <sup>b</sup>	4.86 <sup>b</sup>	0.107	<0.001
CP	4.34 <sup>a</sup>	4.08 <sup>b</sup>	4.03 <sup>b</sup>	0.136	0.004
Starch	6.65 <sup>a</sup>	6.18 <sup>b</sup>	6.15 <sup>b</sup>	0.210	0.001
Apparent total-tract digestibility, %					
DM	66.7 <sup>ab</sup>	68.5 <sup>a</sup>	66.1 <sup>b</sup>	0.66	0.02
OM	67.9 <sup>ab</sup>	69.5 <sup>a</sup>	67.0 <sup>b</sup>	0.64	0.02
NDF	48.0 <sup>a</sup>	47.2 <sup>a</sup>	43.9 <sup>b</sup>	1.07	0.002
ADF	41.1	40.4	42.4	1.54	0.58
CP	67.9 <sup>b</sup>	73.2 <sup>a</sup>	68.8 <sup>b</sup>	0.89	<0.001
Starch	97.6 <sup>ab</sup>	97.8 <sup>a</sup>	97.4 <sup>b</sup>	0.10	0.006

<sup>a,b</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table;  $n = 45$  ( $n$  represents number of observations used in the statistical analysis). Data are presented as LSM.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>Intake during 3-d digestibility and urine data collection periods.

**Table 9.** Nitrogen utilization and purine derivative (PD) excretion in dairy cows fed diets containing canola meal (CM), extruded soybean meal (ESBM), or solvent-extracted soybean meal (SSBM)

Item	Diet			SEM <sup>1</sup>	P-value <sup>2</sup>
	CM	ESBM	SSBM		
N intake, <sup>3</sup> g/d	699 <sup>a</sup>	672 <sup>b</sup>	650 <sup>b</sup>	21.1	0.002
N excretion or secretion, g/d					
Urine N	204	234	220	16.3	0.24
UUN <sup>4</sup>	129 <sup>c</sup>	186 <sup>a</sup>	160 <sup>b</sup>	9.1	<0.001
Fecal N	223 <sup>a</sup>	175 <sup>b</sup>	203 <sup>a</sup>	10.6	<0.001
Total excreta N	427	410	423	20.4	0.67
Milk N	213	207	211	12.1	0.82
As % of N intake					
Urine N	29.8	35.0	34.1	2.14	0.17
UUN	18.8 <sup>b</sup>	27.8 <sup>a</sup>	24.8 <sup>a</sup>	1.14	<0.001
Fecal N	32.0 <sup>a</sup>	26.1 <sup>b</sup>	31.0 <sup>a</sup>	1.06	0.001
Total excreta N	61.8	61.1	65.2	2.47	0.45
Milk N	30.8	30.9	32.6	1.23	0.42
Urine output, kg/d	20.5 <sup>b</sup>	24.5 <sup>a</sup>	24.0 <sup>a</sup>	1.52	0.003
Urinary PD excretion, mmol/d					
Allantoin	657	701	712	76.3	0.86
Uric acid	77.3	77.6	78.0	6.32	0.99
Total PD	809	862	875	91.6	0.85
Creatinine, mg/L	885 <sup>a</sup>	730 <sup>b</sup>	774 <sup>b</sup>	56.9	0.003

<sup>a-c</sup>Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Largest SEM published in table; n = 45 (n represents number of observations used in the statistical analysis). Data are presented as LSM.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>Intake during 3-d digestibility and urine data collection periods.

<sup>4</sup>UUN = urinary urea nitrogen.

some nutritional models likely underestimates MP supply when CM is fed to lactating dairy cows. The NRC (2001) dairy model, for example, uses 36.7% RUP (CP basis) for mechanically extracted CM versus 43% to 48% for SSBM, when DMI is at 4% of BW and the diet is 50% forages. Analyzed RUP in the current experiment, however, was 63.2% versus 49.2% for the 2 meals, respectively. The more recent beef NRC (2016) model ranks RUP of CM higher than that of high-protein SSBM (42.3 versus 29.5%, respectively), which is in the same direction as analyzed data from the current experiment. In contrast, the latest Institut National de la Recherche Agronomique model (Nozière et al., 2018) uses rumen ED of N (calculated at 6%/h passage rate) of 69 and 63% for rapeseed meal and 48% CP SBM (both <5% oil), respectively, which is considerably different from in situ ED of CP determined in the current study (36.7% and 50.6%, respectively, estimated at the same, 6%/h, rate of passage). It is noted that the RUP values for CM estimated in the current experiment are higher than reported by others (e.g., Maxin et al., 2013b; Broderick et al., 2016) and may be specific to the particular batch of CM used in the experiment.

These discrepancies in RUP estimates among nutritional models and analyzed samples can be partially related to variations in the composition of oilseed meals due to cultivar, environmental conditions during growth

and harvest, and meal processing (Paula et al., 2017), but will also depend on the analytical procedures used. In the case of CM, the process of oil extraction from canola seeds is likely also a factor contributing to the higher analyzed versus NRC (2001) values for RUP. Because canola seeds have greater oil content than soybeans (approximately 42 versus 19%, respectively; NRC, 2001), canola requires an extra step to extract the oil from the seeds: first the seeds go through an extrusion process to reduce the oil content to around 18%, after which the meal is subjected to solvent extraction (Canola Council of Canada, 2020). Oil from soybeans is directly extracted with solvent (to produce SSBM) because of their lower oil content. Mechanical extraction, or extrusion, generates heat which decreases ruminal protein degradability and increases RUP content of the meal (Björck and Asp, 1983; Giallongo et al., 2015). As a result CM had a RUP value similar to that of ESBM in the current experiment.

In the present experiment, diets were initially, before the experiment began, formulated using NRC (2001) feed library values for protein fractions for all feed ingredients. However, values presented in Table 1 were derived based on analyzed ingredient composition at the end of the experiment and using values from the conducted in situ experiment for the protein meals. Greater RUP values, lower degradation rates of frac-

tion  $b$  CP, and lower ED of the meals in comparison with NRC (2001) library values resulted in differences between predicted and actual RDP and digestible Met supply in all 3 diets. Although the reconstituted diets suggested deficiencies (based on NRC, 2001) in RDP supply, MUN values for all 3 diets (average of  $10.5 \pm 0.40$  mg/dL) were within the range considered optimal for Holstein cows (i.e., 8 to 12 mg/dL; Kohn et al., 2002), suggesting positive ruminal CP balance. It has been discussed in the literature that NRC (2001) may overpredict RUP supply (Broderick et al., 2010; White et al., 2017), thus underestimating RDP supply in some dietary situations (Cyriac et al., 2008). The in situ technique is the standard technique to predict protein degradability of feedstuffs; however, predictions of  $k_p$  and  $k_d$  appear to be biased in the current NRC (2001) system, resulting in discrepancies between predicted and observed production responses (White et al., 2017). Rumen-protected Met was supplemented to all diets in an attempt to meet NRC (2001) recommendations for digestible Met supply. Differences between initially formulated and reconstituted diet composition, however, resulted in digestible Met deficiencies for all 3 diets, with the ESBM diet having the largest deficiency (0.27 percentage units; intended versus observed Met supply).

### Feed Intake and Milk Production and Composition

Increased DMI when CM replaces SSBM has been previously reported (meta-analyses by Huhtanen et al., 2011; Martineau et al., 2013). It is speculated that the increased DMI in cows fed CM is due to better supply of EAA (i.e., Met), that can potentially enhance MY, and as a consequence, cows would need to increase their DMI to support the increased energy demand of the mammary gland (Huhtanen et al., 2011). Paula et al. (2020) reported increased MY when CM replaced SSBM, but no effect on DMI was observed. Shingfield et al. (2003) also observed marginal responses in milk and ECM yields when heat-treated rapeseed expeller meal replaced SBM with no effects on DMI. In contrast, other studies observed a lack of effect of CM on both DMI and cow performance (Paula et al., 2018; Toti et al., 2018). In the current experiment, the effect of CM on MY was clearly a result of increased DMI, because feed efficiency was similar among diets, which is in agreement with data from Pereira et al. (2020). Increased DMI with the CM diet observed in the current experiment could be partially attributed to differences in the energy density of the diets. Greater DMI was reported when cereal grains were replaced with fibrous by-products (Huhtanen et al., 2008), which was attributed to lower energy density of the latter. In

the present experiment, the CM diet, due to its higher fiber content, had an estimated  $NE_L$  concentration of 1.54 Mcal/kg DM, compared with 1.58 Mcal/kg DM for the SBM diets. Because cows had the same ECM production, lower energy density may have been compensated by greater DMI for the CM diet. The greater intake for the CM diet may be also partially related to greater hay-straw inclusion in the SBM diets (23.5 versus 25.8% in CM and SBM diets, respectively). Rates of passage of forage fiber sources are likely slower than those of nonforage sources (Firkins et al., 1997), which may have influenced rumen fill and therefore DMI.

In the case of ESBM versus SSBM, the difference in MY in the current experiment was partially a result of the numerical increase in DMI by the former diet, likely in addition to its greater RUP content. In our previous studies with ESBM we reported increased DMI (compared with SSBM) in mid-lactation (Giallongo et al., 2015), but not in early-lactation cows (Harper et al., 2019). Diets containing ESBM were also reported to increase MY when replacing SSBM, but no differences in milk components or feed efficiency were observed (Giallongo et al., 2015), which is in agreement with responses in the current experiment. In the meta-analysis by Huhtanen et al. (2011), milk protein yield was increased by CM compared with SBM, which was a result of increased DMI and consequently MY because milk protein concentration was not affected. Similar results were reported in the meta-analysis by Martineau et al. (2013), which is in agreement with data from the current experiment.

Lower MUN concentration in dairy cows fed CM compared with cows fed SSBM is typically reported in the literature (Paula et al., 2018, 2020; Pereira et al., 2020) and is consistent with results from the current experiment. This effect is attributed to lower ruminal protein degradation of CM compared with SSBM (Maxin et al., 2013a), and possibly a better utilization of AA absorbed postruminally in CM-fed cows (Martineau et al., 2014). The increased plasma urea concentration with the SBM diets, compared with CM, agrees with the MUN data in the current study. These results can be partially attributed to the lower intestinal N digestibility (analyzed using a 3-step in vitro technique) of CM, compared with the other treatments. Compared with the other diets, ESBM had a greater digestible RUP supply, which was not accompanied by greater milk protein synthesis, thus likely resulting in increased AA catabolism (Nousiainen et al., 2004) and consequently MUN concentration. In addition, reviews by Santos et al. (1998) and Ipharraguerre and Clark (2005) suggested that microbial efficiency may decrease with increased RUP supply, which could have also contributed to greater MUN values in the ESBM diet. The

greater MUN in SSBM compared with CM diet is likely related to greater RDP supply with the former diet.

### **Plasma Amino Acids Concentrations**

Compared with ESBM, the CM diet had lower total-tract CP digestibility and, accordingly, higher fecal N excretion and lower plasma EAA concentrations. Diets were formulated to provide a similar amount of digestible Met; however, as discussed earlier, differences between formulated and reconstituted diets resulted in different Met supply. The ESBM diet had the largest deficiency in estimated Met supply, as well as the lowest Met plasma concentration. Concentration of an EAA in peripheral blood plasma reflects its absorption and use; hence, if the concentration of an EAA is decreased, it can be considered to be limiting in the diet (Broderick et al., 1974). Further, EAA that are not required by the mammary gland return to peripheral circulation (Arriolo Apelo et al., 2014). Because all 3 diets resulted in similar ECM and milk protein yields in the current experiment, it is likely that greater plasma Met concentration in CM and SSBM, in comparison with ESBM diet, was a result of greater supply of Met that did not result in greater milk protein synthesis. The efficiency of conversion of metabolizable AA into milk protein is not constant among individual AA and varies according to metabolizable AA supply and demand (Doepel et al., 2004). This phenomenon may explain why the CM and SSBM diet did not improve milk protein production, in spite of greater plasma Met concentrations compared with the ESBM diet.

Interestingly, plasma concentration of 1-methylhistidine was decreased by SSBM, compared with the other 2 diets. This response was similar to that observed in a previous experiment (Giallongo et al., 2015), where concentration of plasma 1-methylhistidine was lower for SSBM compared with ESBM processed at 171°C. The mechanism behind this effect is not clear. It is reported that anserine ( $\beta$ -alanyl-1-methyl-histidine) is the precursor of 1-methylhistidine, but it is unknown how hydrolysis of anserine and the subsequent appearance of 1-methylhistidine in blood and urine are regulated in cattle (Houweling et al., 2012).

### **Milk Fatty Acid Proportions**

No differences in milk fat concentration nor yield were observed among diets in the current experiment. However, milk FA composition was clearly altered by the type of protein meal and supplemental oil. It is well documented that the FA profile of milk can be modified by dietary factors (Sutton, 1989; Jenkins and McGuire, 2006). The increased concentration of 18:1,

20:0, and MUFA with CM, compared with the ESBM and SSBM diets, is in agreement with previous reports in which canola oil was added to the diet of lactating cows (DePeters et al., 2001; Hristov et al., 2011a). Similarly, Lopes et al. (2017) observed increased PUFA concentration in milk fat from cows fed conventional ESBM compared with milk from cows fed high-oleic ESBM or whole roasted soybeans.

### **Enteric Gas Emissions**

Treatment did not affect daily enteric CH<sub>4</sub> emission in the current experiment, but cows fed CM produced less enteric CH<sub>4</sub> per kg of DMI (i.e., CH<sub>4</sub> yield) in comparison with both ESBM and SSBM. Gidlund et al. (2015) observed a numerical trend for decreased CH<sub>4</sub> yield in diets with inclusion of heat-treated CM compared with SSBM and associated this reduction to lower CP degradability of the heat-treated CM, which reduced availability of fermentable substrate in the rumen. The decreased CH<sub>4</sub> yield with the CM diet in the current experiment was most likely a result of similar daily CH<sub>4</sub> emission and increased DMI compared with ESBM and SSBM diets. The increase in DMI by CM, however, did not produce a significant effect on ECM and, therefore, CH<sub>4</sub> emission intensity was not affected.

### **Apparent Total-Tract Digestibility and Nitrogen Utilization**

Paula et al. (2018) reported lower DM, OM, NDF, and CP digestibilities in diets in which SSBM was substituted by CM. Other studies reported similar (Brito and Broderick., 2007; Huhtanen et al., 2011) or decreased (Pereira et al., 2020) DM, OM, and CP digestibilities for CM in comparison with SSBM diets. In our previous studies with ESBM we did not observe differences in apparent digestibility of any nutrient between ESBM (171°C extrusion temperature, as in the present experiment) and SSBM diets (Giallongo et al., 2015; Harper et al., 2019). The statistically significant effect of treatment on DM, OM, and starch digestibility in the current experiment was caused by differences between ESBM and SSBM, but digestibility of these nutrients for both diets was similar to that of CM.

The ESBM meal had the greatest (in vitro) intestinal digestibility of CP, which may explain the observed greater CP apparent digestibility of the ESBM diet in comparison with both CM and SSBM diets in the current experiment. However, it is not clear why the SSBM diet had lower NDF digestibility than the CM and ESBM diets. As discussed before, the CM diet had lower portion of NDF coming from forage due to lower hay/straw inclusion compared with the SBM diets.

Nonforage fiber sources have lower iNDF fraction compared with forages (Bhatti and Firkins, 1995), which may have affected NDF digestibility in the SSBM diet, compared with CM. This hypothesis, however, disagrees with the lack of difference in NDF digestibility between the CM and ESBM diets; therefore, it is unlikely that the difference in hay/straw inclusion is the reason for lower nutrient digestibilities in the SSBM diet. Although differences in FA profile among diets exist, the free oil inclusion in the SSBM diet was less than 1% of DM and it is unlikely to have affected fiber degradability.

Urinary creatinine concentration in the current study was lower for the SBM diets, compared with CM and, as a result, estimated urine output was greater for the former diets. Daily creatinine production and consequently creatinine excretion are related to muscle mass and are therefore proportional to the animal's BW (Hobson, 1939; Lofgreen and Garrett, 1954) and are little affected by dietary factors (Chizzotti et al., 2008). On the other hand, urine volume can be affected by N excretion as urine osmolality is kept constant (Bannink et al., 1999). It appears that greater osmotic pressure due to greater plasma urea N concentration with the SBM diets increased the water volume necessary for UUN excretion, resulting in greater urine volume, and by dilution, lower creatinine concentration in spot urine samples. The increased urinary urea excretion for the SBM diets is in agreement with the greater plasma urea and MUN concentrations with these diets compared with CM.

## CONCLUSIONS

In this experiment, RUP content of CM was similar to that of ESBM and considerably greater than RUP of SSBM and NRC (2001) values for CM. This is likely due to heat generated during the extrusion process of CM before solvent extraction. Although CM increased DMI, treatments had no effect on milk components and ECM yield or ECM feed efficiency. Cows fed CM produced less enteric CH<sub>4</sub> per kg of DMI, but had similar CH<sub>4</sub> emission intensity, compared with cows fed the SBM diets, which was a result of the greater DMI and similar ECM yield with the former diet. Overall, data suggest that CM may enhance DMI, but dairy cows fed CM, SSBM, or ESBM, on an equal CP basis, have similar performance in terms of ECM, component yields, and feed efficiency.

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