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Production effects of extruded soybean meal replacing canola meal in the diet of lactating dairy cows

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ABSTRACT

This study investigated the effects of extruded soybean meal (ESBM) in comparison with canola meal (CM) fed on an equivalent crude protein (CP) basis on lactational performance and ruminal fermentation of dairy cows. Following a 2-wk covariate period, 48 Holstein cows averaging (±SD): 146 ± 46 d in milk (DIM) and 43 ± 7 kg/d milk yield (MY) were assigned 1 of 2 treatment diets in a randomized complete block design experiment, which included a 2-wk period for dietary treatment adaptation before experimental data were collected. Following the adaptation period, samples and experimental data were collected for a total of 7 wk. Cows were blocked based on parity, DIM, and MY. Treatment diets contained 15.8% CM (containing 41.2% CP) or 13.2% ESBM (with 48.7% CP) of total mixed ration dry matter (DM), with similar inclusion of other feed ingredients. The CM diet was supplemented with canola oil, whereas the ESBM diet was supplemented with soybean hulls to achieve similar ether extract and neutral detergent fiber contents between the diets. Urea and rumen-protected Met and Lys were added to both diets to meet or exceed cow recommendations. Whole-ruminal digesta samples were collected from 10 (5 per treatment) ruminally cannulated cows. Eight cannulated cows were removed during the last week of the experiment to participate in another study. Treatment did not affect DM intake and MY or energy-corrected MY of the cows. Energy-corrected MY, apart from experimental wk 5, was similar between treatments. Apart from experimental wk 3 and 7, milk fat concentration and yield were greater for cows fed ESBM compared with CM. In multiparous cows only, milk true protein yield was greater for cows fed CM compared with ESBM. Ruminal concentration of total volatile fatty acids and the molar proportion of acetate were greater for ESBM, and propionate and valerate were greater in cows fed CM. Acetate to propionate ratio was greater for cows fed ESBM versus CM diet. Compared with the CM diet, the ESBM diet increased plasma concentrations of Ile, Leu, and Phe but not the sum of essential AA. Apparent total-tract digestibility of acid detergent fiber was greater in cows fed ESBM relative to CM. In this experiment, CM and ESBM included on an equal CP basis in the diet of dairy cows, resulted in similar DM intake, MY, and feed efficiency.
Key words: extruded soybean meal, canola meal, rumen-undegraded protein, dairy cow

INTRODUCTION

By-products of the oilseed industry have been widely used as protein feed ingredients in dairy cattle diets with soybean meal (**SBM**), typically as solvent-extracted SBM (**SSBM**), being the predominantly used oilseed meal. More recently, the increase in production of canola oil in North America has increased availability of canola meal (**CM**), placing it as an alternative to SBM in cattle diets (Martineau et al., 2013). A meta-analysis reported increased milk yield (**MY**; Huhtanen et al., 2011) and other studies reported increased efficiency of N utilization (Broderick et al., 2015; Paula et al., 2018) when CM replaced SBM in dairy diets.

Canola seeds are greater in oil content than soybeans (NASEM, 2021). Thus, a mechanical extraction of oil through an extrusion process has to precede solvent extraction during the production of solvent-extracted CM (Canola Council of Canada, 2021), whereas the lower oil content in soybeans allows a direct solvent extraction for production of SSBM. Heat generated during extrusion typically increases RUP content of oilseed meals (Isenberg et al., 2012; Giallongo et al., 2015).

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The nutritional value and RUP content of CM varies according to growth and harvest conditions and meal processing (Broderick et al., 2016) and several studies (Maxin et al., 2013b; Broderick et al., 2016; Lage et al., 2021) consistently reported greater RUP values for CM (>43% of CP) than values (32% of CP) reported in NASEM (2021). Thus, experimentally determined RUP values for CM are at least 10 percentage units higher than RUP NASEM (2021) values for SSBM (33% of CP), which, combined with experimentally determined greater than NASEM (2021) intestinal digestibility values of CM RUP, would result in greater estimated MP supply by CM compared with SSBM.

Extruded soybean meal (**ESBM**) is produced by pressing soybeans through a die, which increases the temperature of the meal up to 170°C or higher (Björck and Asp, 1983; Giallongo et al., 2015). Previous work demonstrated that increases in meal temperatures increased RUP content in ESBM with consequent benefits in lactational performance of dairy cows when replacing SSBM (Isenberg et al., 2012). In situ studies done in our group have consistently shown that RUP content of ESBM is greater than that of SSBM, ranging from 49 to 63% of CP (Giallongo et al., 2015; Harper et al., 2019; Lage et al., 2021). Considering that experimentally determined RUP content of both CM and ESBM is greater than that of SSBM, it may be more appropriate to compare production responses of replacing CM with ESBM, not SSBM, in dairy diets. However, studies comparing the production effects of ESBM versus CM are scarce. A recent study comparing CM, SSBM, and ESBM included on an equal CP basis, reported greater DMI for CM and similar milk production and feed efficiency between CM and ESBM; however, cows fed SSBM had lower MY when compared with CM and ESBM (Lage et al., 2021). Nevertheless, the Lage et al. (2021) experiment was a short-term crossover design and may not have captured all performance responses (such as BW and BW change effects) to substituting CM with ESBM as a continuous-design experiment would.

The specific objectives of the study were to investigate the effects of CM versus ESBM, included in the diet at equal CP basis on lactational performance, ruminal fermentation, plasma AA profile, nutrient digestibility, and N excretions in mid-lactation dairy cows. Based on results from Lage et al. (2021), we hypothesized that the replacement on an equal CP basis of CM with ESBM would maintain milk production in a long-term experiment and, despite the observed lower DMI (compared with CM), would not have a negative effect on BW gain in lactating dairy cows.

MATERIALS AND METHODS

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

Animals and Diets

The study was conducted with a total of 48 primi- (n = 18) and multiparous (n = 30) Holstein cows, including 10 multiparous ruminally cannulated (10-cm internal diameter cannulas; Bar Diamond Inc., Parma, ID). Primiparous cows averaged (±SD) 150 ± 47 DIM, 567 ± 37 kg BW, and 36 ± 3 kg/d MY, whereas multiparous cows averaged (±SD) 142 ± 41 DIM, 644 ± 56 kg BW, and 47 ± 8 kg/d MY at the end of the covariate period. Cows were housed at The Pennsylvania State University's Dairy Teaching and Research Center freestall barn, equipped with a Calan Broadbent Feeding System (American Calan Inc., Northwood, NH) for monitoring individual cow DMI.

The experiment was a randomized complete block design that lasted a total of 11 wks and had a 2-wk covariate period at the beginning of the study. During the covariate period, cows were fed the following diet (% of DM): corn silage, 37.6; alfalfa haylage, 10.4; grass-hay mixture, 2.4; ground corn grain, 12.0; CM, 12.0; whole roasted soybeans, 8.84; cookie meal, 6.4; whole cottonseed, 4.4; molasses, 4.0; and a mineral and vitamin premix, 2.0. The diet contained (%, DM basis): CP, 16.4; NDF 27.7. The covariate measurement period was followed by a 2-wk dietary treatment adaptation period, and a 7-wk experimental period for data and sample collection. We refer to the weeks during which data and samples were collected as experimental wk 1 to 7, which started on August 21, 2020, and were completed on October 9, 2020. Cows were blocked into 24 blocks of 2 cows each based on parity, DIM, and MY during the covariate period. Cannulation status was also considered as a blocking criterion. Cows had free access to drinking water, and diets were fed as TMR using a Rissler model 1050 TMR mixer (I. H. Rissler Mfg. LLC). Feeding was once daily at approximately 0900 h, and feed was offered for ad libitum intake to approximately 10% refusals. The cows were milked twice daily at approximately 0645 and 1745 h. Eight (4 per treatment) of the rumen-cannulated cows were removed during wk 6 of the experiment to participate in another experiment that required rumen-cannulated animals. These cows were removed after milk composition samples were collected on d 5 of experimental wk 6. Production data (DMI, BW, and MY) for these cows were recorded until cows were removed and were included in the statistical analysis. Thus, production, fecal, urine, and blood data for experimental wk 7 are for only 40 (18 primi- and 22 multiparous) cows. Body condition score and BW change data are for the 40 remaining cows only.

Cows were randomly assigned to 1 of 2 dietary treatments. The ESBM diet included ESBM as the main protein source at 13.2% of dietary DM, whereas the CM diet included CM as the main protein source at 15.8% of dietary DM (Table 1). The ESBM was produced by Fabin Bros. (Indiana, PA) at 163°C extrusion temperature, whereas 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). Our goal was to include CM and ESBM on an equal CP basis, rather than have the same DM inclusion rate. Canola oil (Sam's Club, Bentonville, AR) was added to the CM diet and soybean hulls were added to the ESBM diet to balance for ether extract (**EE**) concentration and NDF contents between diets. Rumen-protected Met (Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) and Lys (AjiPro-L, Ajinomoto Co. Inc., Tokyo, Japan) were added to both diets targeting digestible (**d**)Met and dLys supply of 2.2 and 6.6% of MP, respectively. The daily doses of rumen-protected AA were weighed as a premix and mixed in the TMR. According to the manufacturer's specifications, rumen escape and intestinal digestibility of Met in Mepron were 80 and 90%, respectively, whereas rumen escape and intestinal digestibility of Lys in Ajipro-L were 81 and 91%, respectively. Dietary supplies of dMet and dLys were calculated using DMI of the cows during the covariate period and AA concentration of CM and ESBM (analytical procedures specified below) and NRC (2001) feed library values for all other feed ingredients. Rumen-degraded protein and RUP values for CM and ESBM were determined in an in situ experiment (described below). For RDP and RUP values for all other feed ingredients, NRC (2001) feed library data were used.

Diets were formulated to meet or exceed NE_L requirements and be 5% below MP recommendations, according to NRC (2001). Requirements were calculated for a Holstein cow producing 40 kg of milk/d with 3.50% milk fat and 2.89% true protein at 24.2 kg/d of DMI and BW of 615 kg.

Table 1. Ingredient and nutrient composition of the diets used in the experiment

Item	Diet[1] CM	ESBM
Ingredient, % of DM or as indicated		
Corn silage[2]	36.25	36.25
Alfalfa haylage[3]	14.00	14.00
Straw-hay mixture[4]	4.00	4.00
Cottonseed, whole	6.00	6.00
Corn grain, ground	16.09	16.09
CM	15.80	—
ESBM	—	13.20
Soybean hulls[5]	—	3.40
Molasses[6]	5.00	5.00
Canola oil	0.50	—
Urea	0.48	0.30
Ajipro-L	0.26	0.22
Mepron	0.08	0.13
Mineral mix[7]	1.48	1.48
CP supply from protein meals, g/kg of DMI	65.5	64.5
Nutrient composition, % of DM (or as indicated)		
CP[8]	16.5	16.3
RDP[9]	10.5	9.9
RUP[9]	6.1	6.4
aNDF[8]	29.2	27.9
ADF[8]	19.5	18.4
Ether extract[8]	4.9	4.9
NE_L,[9] Mcal/kg of DM	1.55	1.58
NE_L balance,[9] Mcal/d	3.2	2.0
MP supply,[9] g/d	2,764	3,006
MP balance,[9] g/d	−10	271
Digestible Met,[9] g/d	64	72
Digestible Lys,[9] g/d	189	211
NFC[8]	45.9	46.8
Starch[8]	25.5	26.2
Ca[8]	0.70	0.63
P[8]	0.42	0.32

[1]Average diet composition for adaptation and experimental periods (i.e., experiment wk 1–7). CM = canola meal; ESBM = extruded soybean meal.

[2]Corn silage was 39.9% DM and contained (DM basis) 6.7% CP and 33.5% NDF.

[3]Alfalfa haylage was 32.6% DM and contained (DM basis) 17.5% CP and 42.4% NDF.

[4]Straw-hay mixture was 85.6% DM and contained (DM basis) 10.1% CP and 69.6% NDF.

[5]Soybean hulls were 88.2% DM and contained (DM basis) 12.6% CP and 61.0% NDF.

[6]Liquid molasses from Westway Feed Products (Tomball, TX).

[7]Mineral/vitamin premix (Cargill Animal Nutrition, Cargill Inc., Roaring Spring, PA) contained (%, as-is basis) limestone, 36.75; dry corn distillers grains with solubles, 29.00; NaCl, 24.85; MgO (54% Mg), 4.15; Bio-Phos, 2.45; zinc sulfate, 0.96; mineral oil, 0.5; vitamin E, 0.37; manganese sulfate, 0.37; copper sulfate, 0.26; ferrous sulfate, 0.16; selenite, 0.13; vitamin A, 0.03; vitamin D_3, 0.013; calcium iodate, 0.008; cobalt carbonate, 0.005.

[8]Values calculated using the chemical analysis (Cumberland Valley Analytical Services Inc., Waynesboro, PA) of the feed ingredients and their inclusion in the diets.

[9]Estimated based on NRC (2001) and in situ data from the current experiment for the protein meals using average DMI, milk yield, milk composition, and BW of the cows during the experiment.

Sampling and Measurements

Diet and Feed Ingredients. The amount of feed offered and refused was weighed and recorded daily for each cow at the time of feeding to measure daily as-fed intake during the entire experiment. The weekly DM content of the TMR and refusals were used to calculate TMR DM and consequently, daily DMI for the week. Samples of the concentrate feeds were collected once weekly, and samples of the forages, TMR, and refusals were collected twice weekly and stored at −20°C. Feed samples were later dried for 72 h at 55°C in a forced-air oven and ground in a Wiley mill (Thomas Scientific) through a 1-mm screen for further analyses. Samples were composited (on an equal DM weight basis) to form 1 composite sample for the entire experimental and submitted to Cumberland Valley Analytical Services (Waynesboro, PA) for wet chemistry analyses of CP (method 990.03; AOAC International, 2000), amylase-treated NDF (Van Soest et al., 1991), EE (method 2003.05; AOAC International, 2006), ADF (method 973.18; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), minerals (method 985.01; AOAC International, 2000), and estimated NFC and NE_L. Concentrations of RDP and RUP were determined by using in situ data derived in the current study for CM and ESBM, whereas NRC (2001) values were used for all other feed ingredients. Starch was analyzed as described in Hall (2009). The nutrient compositions of the diets (i.e., CP, NDF, ADF, EE, ash, Ca, and P) were calculated by using the analyzed composition of the individual feed ingredients and their inclusion rate in the TMR (Table 1). Dietary supplies of NE_L, MP, and dMet and dLys were estimated using NRC (2001) based on average DMI, MY, milk composition, and BW of the cows during the experiment.

Samples of CM and ESBM were also analyzed for AA composition (Table 2) 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). Intestinal digestibility of CM and ESBM protein was analyzed at Rock River Laboratories Inc. (Watertown, WI) using the 3-step procedure of Calsamiglia and Stern (1995).

Table 2. Chemical composition and AA concentration (% of DM or as indicated) of canola meal (CM) and extruded soybean meal (ESBM) used in the experiment

	Meal	
Item	CM	ESBM
CP[1]	41.2	48.7
RDP,[2] % of CP	51.3	46.4
RUP,[2] % of CP	48.7	53.6
RUP intestinal digestibility,[3] % of RUP	81.1	99.4
aNDF[1]	29.6	9.90
NDFICP[1,4]	6.30	0.70
ADF[1]	20.4	4.40
ADFICP[1,5]	2.40	0.40
Ash[1]	7.46	7.13
Ca[1]	0.85	0.27
P[1]	1.23	0.68
Ether extract[1]	4.23	8.03
EAA,[6] % CP		
Arg	5.50	6.91
His	2.60	2.53
Ile	4.00	4.56
Leu	6.80	7.48
Lys	5.60	6.02
Met	1.90	1.29
Phe	4.05	5.14
Thr	4.15	3.80
Trp	1.05	1.21
Val	5.02	4.79
Total EAA	40.7	43.7
NEAA,[6] % CP		
Ala	4.22	4.15
Asp	6.55	10.67
Cys	2.47	1.36
Glu	15.92	17.10
Gly	4.87	4.09
Pro	5.72	4.58
Ser	3.37	3.97
Tyr	2.45	3.25
Total NEAA	45.6	49.2
Total EAA and NEAA	86.3	92.9

[1]Analyzed by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) using wet chemistry methods.

[2]Estimated using in situ values and NRC (2001) based on average BW and DMI of cows during the experiment. Passage rates were 7.85 and 7.62%/h for CM and ESBM, respectively.

[3]Analyzed by Rock River Laboratories Inc. (Watertown, WI) using the Calsamiglia and Stern (1995) method.

[4]NDFICP = neutral detergent insoluble crude protein.

[5]ADFICP = acid detergent insoluble crude protein.

[6]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).

Milk Production and Composition. Milk production was recorded daily at each milking. Milk samples were collected from 2 consecutive milkings (p.m. and a.m.) once weekly from experimental wk 3 to 7. Aliquots of the milk sample were placed in tubes with a preservative (2-bromo-2-nitropropane-1, 3-diol) and submitted to Dairy One Cooperative, Inc. (Ithaca, NY) for analysis of milk fat, true protein, lactose, SCC, and MUN using infrared spectroscopy (MilkoScan 4000; Foss Electric, Hillerød, Denmark). Milk composition data were weighed for the corresponding MY during p.m. and a.m. milkings. The averaged MY during each experimental week was used to calculate milk fat, true protein, lactose, and ECM yield. Energy-corrected milk was calculated according to Sjaunja et al. (1990).

Body Weight, BCS, and BW Change. Cow BW was recorded twice daily upon exiting the milking parlor using an AfiFarm 3.04E scale system (S.A.E. Afikim, Rehovot, Israel). Body weight change was calculated as the difference between the average BW during experimental wk 6 and 7 and the average BW during wk 2 of the covariate period divided by days on study. Body condition scoring (Edmonson et al., 1989) was done by the dairy manager both during wk 2 of the covariate period and experimental wk 7. Change in BCS was calculated as the difference between BCS during experimental wk 7 and BCS during wk 2 of the covariate period.

Ruminal Contents. Whole-ruminal digesta and rumen fluid samples were collected from a subset of 10 ruminally cannulated cows (5 on the CM and 5 on the ESBM diets) on d 3 of wk 5 of the experimental period at 0, 4, 8, and 12 h after feeding, as described in Hristov et al. (2011). Briefly, whole-ruminal contents samples collected from 4 locations in the rumen were filtered through 2 layers of cheesecloth. The filtered ruminal fluid was analyzed immediately for pH (59000–60 pH Tester, Cole-Parmer Instrument Company, Vernon Hills, IL), and aliquots were processed and analyzed for VFA (Yang and Varga, 1989) and NH_3 (Chaney and Marbach, 1962) concentrations and total protozoal count (Hristov et al., 2011).

Apparent Total-Tract Digestibility and N Utilization. Spot urine and fecal samples (approximately 300 mL and 500 g per sample, respectively) were collected at 0, 4, 8 and 12 h after feeding for 2 consecutive days during experimental wk 7 for estimation of N utilization and apparent total-tract digestibility of dietary nutrients. A full description of the urine and fecal sample processing and analyses can be found in Lee et al. (2012) and Oh et al. (2013). Aliquots (10 mL) of urine samples were acidified with 0.6 mL of 2 *M* sulfuric acid and diluted 1:10 with 90 mL of distilled water. Acidified urine samples were composited by cow on an equal volume basis and the diluted samples were frozen at −20°C for later analysis of allantoin, uric acid, creatinine, urinary urea N (**UUN**), and total N. Allantoin was analyzed following the procedure by Chen et al. (1992). Stanbio Laboratory (Boerne, TX) kits were used to analyze uric acid (Uric Acid Stanbio Kit 1045), creatinine (Creatinine Stanbio Kit 420), and UUN (Urea Nitrogen Stanbio Kit 580; Stanbio Laboratory Inc., San Antonio, TX). Total N was analyzed in freeze-dried, diluted 1:10 and acidified urine samples using a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). 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 unpublished total urine collection data from Hristov et al. (2011). Daily total N, UUN, and purine derivatives excretions were calculated using the estimated urine output.

Fecal samples were oven-dried at 65°C, ground through 1-mm screen in a Wiley mill (Thomas Scientific), and analyzed for DM, OM, CP, starch, NDF, and ADF. Analysis of OM was conducted by ashing the TMR samples for 4 h at 600°C. A Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) was used to pulverize a 0.5-g aliquot of fecal sample for CP analysis (N × 6.25) using the Costech ECS 4010 C/N/S elemental analyzer. Samples were analyzed for NDF and ADF using an Ankom 200 fiber analyzer (Ankom F57 bags, Ankom Technology Corp., Macedon, NY) based on Van Soest et al. (1991) using α-amylase and sodium sulfite in the NDF analysis. Starch analysis of fecal DM was according to (Hall, 2009). Apparent total-tract digestibility of nutrients was estimated using indigestible NDF as an intrinsic digestibility marker (Schneider and Flatt, 1975). Fecal and TMR samples were analyzed for indigestible NDF after a 12-d ruminal incubation in situ according to Huhtanen et al. (1994), with the exception that 25-μm pore size Ankom filter bags (F57; Ankom Technology, Macedon, NY) were used for the rumen incubation (Lee et al., 2012).

Blood Sampling. Blood samples were collected from coccygeal vein or artery at the same times as the fecal and urine samples for 2 consecutive days during experimental wk 7. Whole-blood samples (approximately 10 mL each) were collected into evacuated tubes containing EDTA (BD Biosciences). Plasma was obtained via centrifugation at 1,500 × *g* at 4°C for 10 min and stored at −20°C until analysis. Plasma samples were composited per cow in an equal volume basis and sent to the University of Missouri-Columbia's Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for free AA analysis following the procedures of Deyl et al. (1986) and Fekkes (1996). A separate set of composited blood plasma samples were analyzed for glucose (Stanbio Glucose LiquiColor, Kit 1070, Stanbio Laboratory Inc.); BUN (Stanbio Urea Nitrogen Kit 0580, Stanbio Laboratory Inc.); plasma total fatty acid (HR Series NEFA-HR, Wako Diagnostics, Richmond, VA); and BHB (Autokit 3-HB Microliter Procedure; Wako Diagnostics).

In Situ Protein Degradability. Ruminal disappearance of CP in CM and ESBM was determined in situ. Three lactating Holstein cows averaging (±SD) 65 ± 12 DIM; 41 ± 1 kg/d MY; 716 ± 21 kg BW and 31 ± 2 kg/d DMI were used for the in situ experiment and were fed the following diet (% of DM): corn silage, 39.4; alfalfa haylage, 12.1; grass-hay mixture, 1.61; ground corn grain, 10.4; SSBM, 8.8; whole roasted soybeans, 8.8; cookie meal, 7.2; whole cottonseed, 4.8; molasses, 4.8; and a mineral and vitamin premix, 2.0. The diet contained (%, DM basis): CP, 16.5; NDF 29.2. The experimental procedures were as described by Lee et al. (2012). Samples of the protein meals were collected throughout the experimental period and composited on an equal weight basis. Five grams of each of the protein meals (as-fed basis and without further processing) were weighed into Ankom polyester bags (10 × 20 cm nylon bags with 50 ± 10 μm porosity; Ankom Technology Corp., Macedon, NY), which were sequentially incubated in the ventral rumen for 0 (processed as the other samples but not incubated in the rumen), 2, 4, 6, 16, and 24 h and simultaneously removed. Bags were incubated in triplicate for each time point and cow. Samples were presoaked in warm (39°C) water for 1 min before being placed into the rumen. Following incubation, all bags were manually washed, including the 0 h samples, with cold tap water until the water ran clean. All samples were oven-dried for 72 h at 55°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); CP was calculated as N × 6.25. The fractional rate of ruminal disappearance of N from the protein meals was derived by fitting the natural logarithm of the fraction of N remaining in the bag over time to a linear regression model (SigmaPlot 13.0; Systat Software Inc., San Jose, CA; $R^2 \geq 0.88 \pm 0.12$):

$$b = a + c \times t,$$

where a (%) is the soluble fraction of CP, b (%) is the fraction of protein meal CP remaining in the bag at time t, and c (%/h) is the rate of degradation of protein meals; $a + b$ was constrained to be ≤100%. In the current experiment, RUP of CM and ESBM was estimated from the in situ CP (N × 6.25) degradability data according to NRC (2001) as: RUP (%) = 100 − RDP; and RDP (%) = soluble CP (%) + [100 − soluble CP (%)] × [rate of CP degradation ÷ (rate of CP degradation + ruminal passage rate)]. Rate of passage used was obtained based on NRC (2001) equations. The in situ effective ruminal degradability (**ED**) of CP was determined using the following equation (Ørskov and McDonald, 1979):

$$\text{ED} = a + b \times [c \div (c + k)],$$

where a, b, and c are as specified above and k is the rate of passage, assumed to be 0.06/h, which is passage rate estimated for concentrate feeds when DMI is at 4% of BW and the diet is 50% forages (NRC, 2001).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS, version 9.4 (SAS Institute Inc., Cary, NC). The animal data were analyzed as a randomized complete block design experiment. Data were tested for normality using the UNIVARIATE procedure and were processed for outlier identification using the REG procedure based on an absolute studentized residual value >3. Log-transformed data were analyzed when the W statistic of Shapiro-Wilk test was less than 0.05 (i.e., SCC and rumen protozoal counts). Production data were averaged by experimental week and the average values were used in the statistical analysis. Measurements from covariate wk 2 were included in the model for all the production variables analyzed. The statistical analysis did not include data collected during the adaptation period. The statistical model for the production data (DMI, MY, ECM yield, milk composition, BW, feed efficiency, and ECM feed efficiency) included the fixed effect of treatment, week, parity, and treatment × week. A treatment × parity interaction was tested in the model and, with exception of milk true protein yield and MUN, was nonsignificant ($P > 0.05$) for all variables, in which case the interaction was removed from the final models. Block and block × treatment were random effects. The individual cow average daily MY and milk compositions during each experimental week were used to calculate yields of milk fat, true protein, lactose, ECM yield, and ECM feed efficiency. These data were analyzed as repeated measures using the AR(1) covariance structure; week was the repeated term and block × treatment was the subject.

Plasma AA profile, BW and BCS change, rumen fermentation, nutrient intake and apparent digestibility, and N utilization and excretion data were analyzed with treatment and parity in the statistical model. Block and block × treatment were random effects, and all others were fixed. Similar to the production data, treatment × parity interaction was included in the model and, with exception of milk N secretion (in g/d, or as % of N intake), was found to be nonsignificant ($P > 0.05$) for all variables and was removed from the final models.

Statistical differences were considered significant at $P \leq 0.05$, and a trend was declared at $0.05 < P \leq 0.10$. Unless indicated otherwise, data are presented as LSM.

RESULTS AND DISCUSSION

Diet and Feed Characteristics

Ingredient and nutrient composition of the diets are presented in Table 1. In this study, our interest was to understand production, plasma AA, ruminal fermentation, apparent total-tract digestibility, and N excretion and utilization responses to an equal CP replacement of CM with ESBM in the diet of lactating cows. Sub-

Table 3. Ruminal in situ degradability of CP in canola meal (CM) and extruded soybean meal (ESBM)[1]

Item	Meal			
	CM	ESBM	SEM[2]	*P*-value
Soluble fraction (*a*), %	29.9	27.2	0.22	0.001
Potentially degradable fraction (*b*), %	70.1	72.8	0.22	0.001
Rate of degradation of *b* (*c*), %/h	3.43	2.73	0.053	0.007
Effective degradability,[3] %	55.4	50.2	0.32	0.003

[1]The fractional rate of ruminal degradation of CP in protein meals was derived by plotting the natural logarithm of the fraction of meals remaining in the bag over time and fitting the data to a linear regression model (SigmaPlot 13.0; Systat Software Inc., San Jose, CA; $R^2 \geq 0.88 \pm 0.12$).

[2]Largest SEM reported in table; n = 6 (n represents the number of observations used in the statistical analysis).

[3]Esimated using the equation of Ørskov and McDonald (1979): effective degradability = $a + b \times [c \div (c + k)]$, where a, b, and c are as specified above, and k is the rate of passage assumed to be 6%/h.

stitution studies of this nature are challenging given that the addition or exchange of more than one dietary ingredient is often required to account for inherent differences between feeds. These substitution decisions can affect overall animal response beyond effects of the primary feeds of interest. For the current study, we decided to supplement the CM diet with canola oil and the ESBM diet with soybean hulls to achieve similar EE and NDF contents between the diets. Inclusion of protein meals was kept on an equal CP basis between diets. Dietary treatments were originally formulated to be slightly below MP requirements of the cows (5% MP deficiency) at the beginning of the experiment using covariate measurements. To achieve this MP target while avoiding the dietary inclusion of another protein feedstuff, we included urea in both diets. In addition, as mentioned above, rumen-protected Met and Lys were supplemented to ensure that AA deficiencies would not limit production responses. Metabolizable protein and NE_L balances were estimated using NRC (2001) at the end of the experiment based on DMI and production of the cows during the experiment. The intended 5% MP deficit was not achieved for either diet (Table 1) due to a greater-than-anticipated DMI (8.2% greater, on average) and a decrease in milk production by cows during the experiment relative to when diets were formulated. According to NRC (2001), RUP digestibility for the ESBM diet was approximately 10 percentage units greater when compared with the CM diet (89.7 vs. 79.9% of RUP, respectively; NRC, 2001) and agrees with the analyzed greater intestinal digestibility of ESBM when compared with CM (Table 2). It should be noted that intestinal digestibility of RUP for ESBM was high in the current experiment. Furthermore, intestinal digestibility of RUP of ESBM used in Lage et al. (2021) was greater than that of SSBM, which suggests that the extrusion process may increase digestibility of RUP in the small intestine.

The ESBM used in the current experiment had 7.5 percentage units greater CP concentration than CM (Table 2). The concentration of RUP in both CM and ESBM was greater than mean NASEM (2021) values for these feeds (32.0 and 45.0% of CP, respectively). It should be noted, however, that comparing RUP values for protein feeds among experiments and nutritional models is difficult. For example, NASEM (2021) uses 5.5 and 10%/h passage rates for calculating RUP of CM and ESBM, respectively, whereas passage rates of 7.9 and 7.6%/h (based on NRC, 2001, and average BW and DMI data of cows during the experiment) were used for estimating RUP for CM and ESBM in the current experiment. As mentioned above, intestinal digestibility of RUP, analyzed using the 3-step procedure of Calsamiglia and Stern (1995), was greater for ESBM when compared with CM. Canola meal was approximately 3-fold greater in aNDF content than ESBM. Concentrations of Arg, Ile, Leu, Lys, Phe, and Trp were greater, whereas those of His, Met, Thr, and Val lower, for ESBM in comparison with CM. Concentrations of Cys, Gly, and Pro were greater for CM compared with ESBM. Together, concentrations of total EAA and EAA + NEAA were approximately 8% greater for ESBM when compared with CM. Amino acid composition of the protein meals was comparable to table values (NASEM, 2021).

In situ CP degradability characteristics for CM and ESBM are presented in Table 3. The soluble fraction (*a*) and rate of degradation of the potentially degradable fraction (*b*) of CP were greater ($P \leq 0.001$) for CM, whereas the potentially degradable fraction (*b*) of CP was greater ($P = 0.001$) for the ESBM. In situ ED was greater ($P = 0.003$) for CM when compared with ESBM.

Soluble (*a*) and potentially degradable (*b*) N fractions values for CM in the current experiment were similar to those reported by Broderick et al. (2016) for

CM samples averaged across 4 production years. When estimated using NRC (2001), RDP was approximately 4 percentage units lower for CM used in the current experiment relative to the values reported in Broderick et al. (2016). On the other hand, the RUP content of CM in the current experiment was approximately 14 percentage units lower than values reported in Lage et al. (2021) and resembled values reported by Maxin et al. (2013a) and Broderick et al. (2016). Rumen-undegraded protein of ESBM (extruded at 163°C) used in the current experiment was 4 percentage units greater than RUP of the ESBM (extruded at 160°C) used in Harper et al. (2019) but 5 percentage units lower than that of ESBM extruded at a higher temperature (171°C) used in Giallongo et al. (2015). The RUP concentration of ESBM used in the current experiment, along with values reported for ESBM elsewhere (Giallongo et al., 2015), confirm that RUP content of soybean meal linearly increases with extrusion temperature (Isenberg et al., 2012). Consequently, in situ ED of ESBM in the current experiment was 14 percentage units lower than ESBM extruded at a higher temperature used in Giallongo et al. (2015).

Solvent-extracted CM is a common feed used in dairy rations; however, a mechanical extraction of the oil through an extrusion process must precede solvent extraction during the production of solvent-extracted CM (Canola Council of Canada, 2021). Likely as a result of processing, research to date (Maxin et al., 2013b; Lage et al., 2021) has shown that RDP and RUP of CM may be incorrect in NRC (2001), which results in underestimation of MP supply when CM is included in diets of lactating dairy cows. This is in line with observations from the current experiment where estimated RUP values for CM were greater than the average values in NRC (2001) for mechanically extracted CM (48.7 vs. 36.7% of CP, respectively). Additionally, estimated RUP values for CM were also greater than average values in NASEM (2021) for solvent-extracted CM (48.7 vs. 32.0% of CP, respectively). Processing, growth, and harvest conditions affect the nutritional value of CM (Broderick et al., 2016), which likely explains why experimentally determined RUP is greater than RUP values reported for CM in NRC (2001) and NASEM (2021). In terms of rumen escape protein supply, in situ estimates of ruminal degradability of CP in the current study suggest that comparison of CM with ESBM is more appropriate than with SSBM. Here, it should be noted that issues such as lack of chewing, assumptions about retention time, physical loss of feed particles through the bag pores that may escape ruminal fermentation, and inaccurate quantification of microbial contamination of undigested residues can all affect in situ estimates (Huhtanen and Ahvenjärvi, 2022). Thus, NRC (2001) estimates for protein degradability are often criticized, mostly due to issues related to the in situ procedure used to derive RDP and RUP values (Huhtanen and Hristov, 2009); however, in the current experiment, according to NRC (2001), the ESBM diet supplied RUP at a 22% excess of cows' requirements, whereas the CM diet was slightly (0.7%) deficient in RUP. Both ESBM and CM diets met and exceeded, respectively, the RDP requirements, according to NRC (2001). Digestible Met and dLys supplies were adequate for both diets (>2.2 and 6.6% of MP, respectively; Schwab et al., 2005) and greater for ESBM relative to CM diet. Supply of NE_L was approximately 8 and 5% above cow requirements for the CM and ESBM diets, respectively.

Dry Matter Intake and Milk Production

Dry matter intake was similar between treatments throughout most of the experiment (Figure 1), but there was a treatment × week interaction ($P < 0.001$) caused by higher DMI for the ESBM diet during experimental wk 3. Reexamination of the raw DMI data did not offer a clear explanation for this difference, thus the intake effect for experimental wk 3 and, consequently, the interaction effect was considered an aberration. Overall, replacement of CM with ESBM in the diet did not affect DMI, MY, or ECM yield, and no effect of treatment was observed on feed efficiency (Table 4). However, numerical differences in DMI and ECM between treatments caused a treatment × week interaction ($P = 0.004$) for ECM feed efficiency; it was greater for cows fed the ESBM diet during experimental wk 5 (Figure 2). Regardless of treatment, multiparous cows used in the current experiment had greater ($P < 0.01$) feed efficiency relative to primiparous cows, which is in line with data reported by others (Linn et al., 2004).

A meta-analysis reported increased DMI (Huhtanen et al., 2011), whereas more recent studies reported increased MY (Paula et al., 2020; Lage et al., 2021) when CM replaced SSBM in diets of lactating dairy cows. Studies comparing CM with ESBM in dairy diets are scarce. Giallongo et al. (2015) reported increased DMI and MY when ESBM replaced SSBM at 13% of dietary DM in mid-lactation dairy cows. Productive responses to ESBM inclusion in the diet of lactating dairy cows have been more variable, with Harper et al. (2019) reporting no increases in DMI and MY in early-lactation cows when ESBM replaced SSBM. Furthermore, Harper et al. (2019) did not report a treatment × week effect when ESBM replaced SSBM in diets of early-lactation cows. To our knowledge, this is the first experiment investigating the effect of treatment × week in cows fed diets replacing CM with ESBM. Clearly,

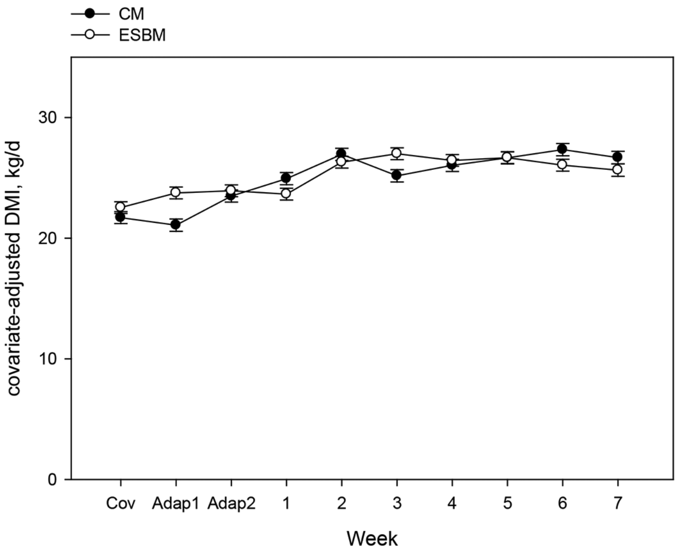

Figure 1. Dry matter intake of dairy cows fed diets with inclusion of canola meal (CM) or extruded soybean meal (ESBM). Data from covariate wk 2, designated as Cov, are LSM, and error bars represent SEM. All other data are covariate-adjusted LSM. Following the covariate measurement period, the first 2 wk of feeding the treatment diets were allowed for adaptation and are designated as Adap1 and Adap2, respectively. Following the adaptation period, data and samples for statistical analysis were collected for a total of 7 experimental weeks. Main effect of treatment: $P = 0.67$. Treatment × week: $P < 0.001$. Eight cows (4 per treatment) were removed to participate in another experiment during experimental wk 7.

with exceptions of experimental wk 3, DMI was similar between treatments throughout the experiment. More recently, cows fed ESBM did not show differences in ECM, feed efficiency and ECM feed efficiency when compared with cows fed CM (Lage et al., 2021). However, in that experiment, cows fed the CM diet had greater DMI than cows fed the ESBM diet, which the authors attributed to differences in energy density and forage fiber content between diets. In addition, in the experiment by Lage et al. (2021), CM and ESBM were deficient in Met, with the ESBM diet having the largest deficiency. Huhtanen et al. (2011) proposed that replacement of soybean protein meals with CM in the diet of lactating cows typically resulted in increased nutrient supply, particularly dMet, which potentially increased MY and induced greater DMI as a necessary response to greater energy demands. However, results from the current experiment suggest that equal CP replacement of CM with ESBM elicit a similar response in terms of production performance when dietary MP, Met, and Lys supplies are adequate.

Milk Composition and BW

Milk fat concentration and yield were greater ($P \leq 0.02$) in cows fed ESBM relative to CM (Table 4), except on experimental wk 3 and 7 (treatment × week interaction, $P \leq 0.03$; Figure 3). No difference between treatments was observed for milk true protein concentration. However, in multiparous cows only, milk true protein yield was greater ($P = 0.05$) by 70 g/d, whereas MUN ($P < 0.01$) was lesser when cows were fed CM, relative to ESBM. In primiparous cows, MUN concentration was not different between treatments, but milk true protein yield tended to be lesser ($P = 0.09$) in cows fed CM in comparison with ESBM. Dietary treatments did not affect milk lactose concentration and yield. Body weight, BCS, and BCS and BW change were not affected by treatments and no treatment × parity interactions were observed.

A meta-analysis showed no response in milk fat when CM substituted SBM in diets of lactating dairy cows (Martineau et al., 2013), whereas the existing literature on the influence of a heat-treated SBM on milk fat is equivocal. Previous research conducted in our laboratory where ESBM replaced SSBM in lactating cow diets showed no effect on milk components and component yields (Giallongo et al., 2015; Lage et al., 2021). Conversely, increased milk fat concentration and yield were observed in a study where a heat-treated SBM replaced SSBM in the diet of lactating cows (Broderick et al., 2009). It should be emphasized that several experiments to date have investigated milk production and composition in response to extruded full-fat soybeans, whereas others focus on expeller SBM (Socha, 1991). Extruded SBM is typically lower in EE and greater in CP contents than extruded full-fat soybeans but greater in EE and lower in CP than expeller SBM (Harper et al., 2019). Together, differences in EE and CP content found in the different types of heat-treated SBM can affect milk components and components yields. Several factors could have collectively induced the increases in milk fat concentration and yield for cows fed the ESBM diet in the current experiment. The addition of soybean hulls, a feed that is high in digestible NDF and is fermented predominantly to acetate in the rumen (Ipharraguerre et al., 2002) could partially explain the milk fat effect of the ESBM diet. On the other hand, extrusion of oilseeds may protect UFA from ruminal biohydrogenation (Sterk et al., 2012) by denaturing the protein matrix surrounding the fat cells, increasing the extent of rumen escape, and consequently reducing rumen availability of UFA (Kennelly, 1996). Another mechanism suggested is that the extrusion process can potentially bind lipids to starch and protein (Björck and Asp, 1983) and increase the oil holding capacity of soybean meal (Singh and Koksel, 2021). This could have also led to a greater ruminal bypass of UFA in the ESBM diet relative to the free oil supplemented in the CM diet (Chouinard et al., 1997a). Greater rumen

Table 4. Dry matter intake, milk production and composition, BW, and body condition of dairy cows fed diets containing canola meal (CM) and extruded soybean meal (ESBM)[1]

Item	Treatment[2] CM	ESBM	SEM[3]	P-value[4]
DMI, kg/d	26.2	25.9	0.46	0.67
Milk yield, kg/d	39.2	38.5	0.59	0.24
Feed efficiency,[5] kg/kg	1.48	1.49	0.023	0.71
Milk fat, %	3.51	3.95	0.094	0.003
Yield, kg/d	1.33	1.47	0.048	0.02
ECM,[6] kg/d	35.8	36.6	0.90	0.31
ECM feed efficiency,[7] kg/kg	1.37	1.40	0.035	0.38
Milk true protein, %	3.18	3.19	0.049	0.93
Yield, kg/d				
Primiparous	1.06	1.12	0.037	0.09
Multiparous	1.27	1.20	0.032	0.05
Milk lactose, %	4.80	4.85	0.020	0.07
Yield, kg/d	1.86	1.83	0.04	0.47
MUN, mg/dL				
Primiparous	11.8	12.0	0.28	0.58
Multiparous	10.6	11.8	0.23	0.001
SCC,[8] $\times 10^3$ cells/mL	100.4	43.8	22.0	0.36
BCS[9]	3.05	3.01	0.07	0.69
BW, kg	636.2	632.3	3.82	0.27
BCS change[10]	0.07	−0.10	0.081	0.11
BW change,[11] g/d	551	713	131.8	0.40

[1]Milk samples for composition analysis were collected from experimental wk 3 to 7. Milk composition data were from 40 cows only during experimental wk 7.

[2]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[3]Largest SEM reported in table; n = 320 for DMI, milk yield; n = 274 for BW; n = 320 for feed efficiency; n = 225 for milk composition and ECM data; n = 40 for BCS and BW change (n represents the number of observations used in the statistical analysis).

[4]Main effect of treatment. Treatment × week interaction, $P \geq 0.09$, except for DMI ($P < 0.001$), ECM feed efficiency ($P = 0.004$), and milk fat concentration ($P = 0.03$) and yield ($P = 0.02$). Treatment × parity (primi- vs. multiparous) interaction, $P > 0.07$, except for milk true protein yield ($P = 0.01$) and MUN ($P = 0.05$).

[5]Milk yield ÷ DMI.

[6]ECM (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).

[7]ECM yield ÷ DMI.

[8]Statistical analysis was performed on log-transformed data.

[9]BCS. Edmonson et al. (1989).

[10]BCS change was: (average BCS during experimental wk 7 − average BCS covariate wk 2).

[11]BW change: (average BW during experimental wk 7 − average BW covariate wk 2) ÷ days on study.

escape UFA would likely result in lesser detrimental effect on fiber-degrading bacteria in the rumen and is in line with the increased molar proportion of acetate and total-tract digestibility of ADF observed for ESBM-fed cows in the current experiment (discussed below). Additionally, long-chain fatty acids (**FA**) in milk fat are predominantly derived from dietary sources (Palmquist and Jenkins, 1980) and increasing the postruminal supply of long-chain FA has been previously shown to increase milk fat in Holstein cows (Lopes et al., 2017). Thus, it is also possible that the observed increase in milk fat with the ESBM diet in the current experiment is due to decreased ruminal but greater postruminal availability of FA in ESBM due to the extrusion process (Chouinard et al., 1997b). It has also been shown that greater availability of dietary UFA may affect milk fat synthesis in the mammary gland (Bauman et al., 2011). Therefore, although canola oil was supplemented to the CM diet at a relatively low level, it is possible that it contributed, through ruminal mechanisms, to differences in milk fat concentration and yield between treatments in the current experiment.

The meta-analyses by Huhtanen et al. (2011) showed that milk true protein concentration did not increase in CM diets relative to SSBM; however, in Martineau et al. (2013), milk true protein yield was increased as a result of increased MY in cows fed diets formulated with CM. When SSBM was replaced by ESBM in diets of early- and mid-lactation dairy cows, Harper et al. (2019) and Giallongo et al. (2015) did

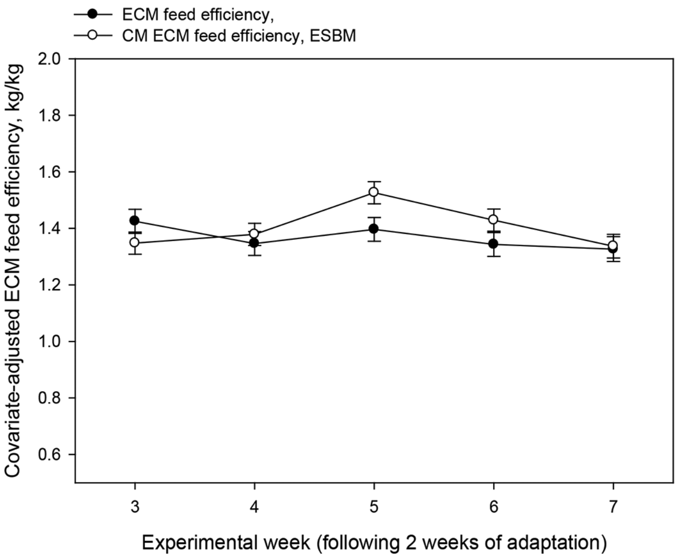

Figure 2. Energy-corrected milk feed efficiency (i.e., kg of ECM/kg of DMI) of dairy cows fed diets with inclusion of canola meal (CM) or extruded soybean meal (ESBM). Data are covariate-adjusted LSM, and error bars represent SEM. Main effect of treatment: $P = 0.38$. Treatment × week: $P = 0.004$. Cows were allowed a 2-wk period for adaptation to dietary treatments before the beginning of a 7-wk period of data and sample collection (i.e., experimental wk 1–7). Eight cows (4 per treatment) were removed to participate in another experiment during experimental wk 7.

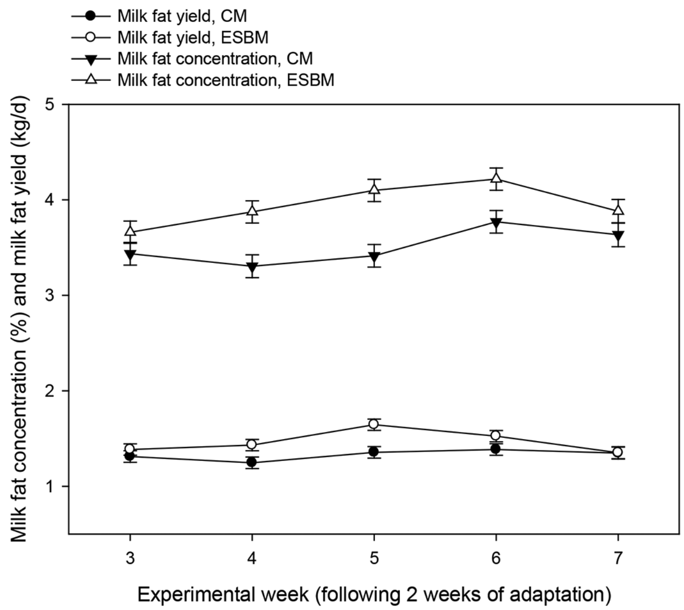

Figure 3. Milk fat concentration and yield of dairy cows fed diets with inclusion of canola meal (CM) or extruded soybean meal (ESBM). Data are covariate-adjusted LSM, and error bars represent SEM. Main effect of treatment: $P \leq 0.02$ for both variables. Treatment × week: $P \leq 0.03$ for both variables. Cows were allowed a 2-wk period for adaptation to dietary treatments before the beginning of a 7-wk period of data and sample collection (i.e., experimental wk 1–7). Eight cows (4 per treatment) were removed to participate in another experiment during experimental wk 7.

not observe differences in milk true protein concentration and yield. Similarly, Lage et al. (2021) reported no difference in milk true protein concentrations and yields among lactating cows fed diets containing CM, ESBM, or SSBM. However, as mentioned above, all diets in Lage et al. (2021) were deficient in dMet, which could have affected their results. In the current study, because milk true protein concentration was similar between treatments, the greater milk true protein yield in multiparous cows fed CM was likely a result of numerically greater MY (41.5 vs. 40.6 kg/d, respectively), which was not observed for primiparous cows (34.6 vs. 34.3 kg/d, respectively). Previous research has shown that increased MY increases efficiency of N utilization (Huhtanen and Hristov, 2009) which agrees with N excretion and secretion data reported below. Differences in milk protein in the current experiment resulted in greater milk N secretion (in g/d and as % intake) in multiparous cows fed the CM relative to the ESBM diet discussed below.

When compared with multiparous cows fed the CM diet, the greater MUN concentration in multiparous cows fed the ESBM diet is likely a result of the greater RUP supply with the latter diet and, as discussed above, possibly reflects decreased utilization efficiency of RUP for milk protein synthesis. This aligns with Broderick et al. (2009) who suggested that greater dietary RUP concentrations lead to elevated MUN and UUN (discussed below) when milk true protein yield is not altered. Similar to results in the current experiment, Lage et al. (2021) reported greater MUN concentration in cows fed the ESBM diet when compared with CM. In that experiment, authors suggested that greater RUP supplies with ESBM without a corresponding increase in milk protein synthesis likely resulted in increased AA catabolism and MUN concentration (Nousiainen et al., 2004).

Ruminal Fermentation

Data for ruminal fermentation variables in the current experiment are presented in Table 5. Ruminal pH was not affected by treatment. Total VFA concentration, however, was increased ($P = 0.01$) 17% by the ESBM diet when compared with CM. Molar proportion of acetate ($P = 0.05$) was increased, whereas proportions of propionate and valerate were decreased ($P \leq 0.03$) in ruminal fluid of cows fed ESBM relative to CM. Consequently, acetate to propionate ratio was greater ($P = 0.006$) for cows fed ESBM than CM. There was no effect of dietary treatment on NH_3 concentration. Protozoal counts were greater ($P = 0.002$)

Table 5. Rumen fermentation parameters of dairy cows fed diets containing canola meal (CM) and extruded soybean meal (ESBM)[1]

Item	Treatment[2] CM	ESBM	SEM[3]	*P*-value[4]
pH	6.12	6.10	0.087	0.92
Total VFA, m*M*	111	130	4.2	0.01
VFA (mol %)				
Acetate	56.3	60.3	1.19	0.05
Propionate	26.3	23.2	0.96	0.01
Butyrate	10.9	11.7	0.81	0.52
Isobutyrate	0.87	0.88	0.065	0.84
Valerate	3.70	2.11	0.351	0.03
Isovalerate	1.86	1.84	0.091	0.89
Acetate:propionate	2.18	2.65	0.129	0.006
NH_3, m*M*	7.85	9.68	0.966	0.22
Total protozoa,[5] $\times 10^4$/mL	8.10	10.9	0.35	0.002

[1]Whole ruminal contents were collected during experimental wk 5 for rumen fermentation analyses.

[2]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[3]Largest SEM reported in table; n = 40 (n represents the number of observations used in the statistical analysis).

[4]Main effect of treatment.

[5]Actual protozoal counts were log-transformed for the statistical analysis.

for cows fed the ESBM diet when compared with CM-fed cows.

Previous experiments have not reported differences in ruminal total or individual VFA concentrations when CM (Brito and Broderick, 2007; Paula et al., 2018) or ESBM (Giallongo et al., 2015) replaced SSBM in the diet of lactating dairy cows. In the current study, the greater total VFA concentration suggests increased microbial activity and OM degradability in the rumen of cows fed ESBM, compared with CM. The reported increase in the molar proportion of acetate and acetate:propionate ratio in ruminal fluid of cows fed ESBM is in line with the observed increases in milk fat concentration and yield with that diet. Earlier studies have proposed that the high concentration of rapidly fermentable NDF in soybean hulls enhance ruminal fermentation while increasing molar proportions of acetate (Ipharraguerre et al., 2002; Ipharraguerre and Clark, 2003). It is also possible that addition of canola oil in the CM diet could have affected ruminal microbe proliferation (Maia et al., 2007) by increasing the UFA load leading to reductions in fiber degradation. The 2 diets had similar EE concentration, but due to reasons mentioned above, it is possible that FA in ESBM were not equally available for ruminal fermentation when compared with FA from canola oil in the CM diet. Furthermore, Giallongo et al. (2015) reported increased populations of fibrolytic rumen bacteria (*Fibrobacter* and *Fibrobacterales* spp.) in ruminal contents of cows fed ESBM extruded at higher (171°C) versus lower (149°C) temperatures suggesting a possible effect of extrusion temperature on ruminal fermentation. The greater molar proportion of valerate in ruminal contents of cows fed CM in the current study agrees with results in Paula et al. (2017) and Broderick et al. (2015) when CM replaced SSBM in vitro and in diets of lactating dairy cows, respectively. However, in those experiments CM was a greater source of RUP than SSBM, whereas the opposite was true in the current experiment. The greater molar proportion of valerate in ruminal fluid of cows fed the CM diet in the present study could be attributed to the higher RDP content of CM versus ESBM and catabolism of feed AA in the rumen (Van Soest et al., 1991; Broderick et al., 2015). In support of this, it has been proven that valerate can form from the degradation of AA such as Arg, Pro, and Lys (El-Shazly, 1952).

When compared with CM, cows fed the ESBM diet had greater protozoal population. Earlier reports have shown that ruminal protozoa can decrease the rate of starch degradation in the rumen by ingesting starch granules and to possess fibrolytic and proteolytic activities (Williams and Coleman, 1997). Thus, greater protozoa population in the ruminal contents of cows fed ESBM helps explain the increased acetate:propionate ratio in that diet, compared with CM. Furthermore, it has been demonstrated that dietary UFA negatively affect ruminal protozoa (Ivan et al., 2013), which is in line with our previous discussion about the greater ruminal availability of canola oil, relative to oil in ESBM.

Plasma AA and Metabolites

Compared with CM, cows on the ESBM diet had greater ($P \leq 0.03$) plasma concentrations of Ile, Leu, and Phe (Table 6). Plasma concentration of Met, Lys, and His were similar between treatments. The ESBM-fed cows had greater ($P = 0.05$) plasma concentration of Asp, tended to have an increased ($P = 0.06$) concentration of Asn and decreased ($P = 0.003$) concentration of Glu relative to the CM diet. Overall, the sum of EAA, NEAA, and total AA in plasma were similar between cows fed the 2 diets. The ESBM diet had elevated ($P = 0.002$) plasma concentrations of 1-methyl-histidine (**MH**) when compared with the CM diet. No difference was observed in plasma concentrations of 3-MH, urea, and carnosine. Plasma metabolites, apart from an increase in blood glucose concentration ($P = 0.001$) in cows fed the CM diet, were not affected by treatment (Table 7).

According to NRC (2001), the diets fed in the current experiment supplied sufficient amounts of dMet

Table 6. Blood plasma AA concentrations (µ*M*) in dairy cows fed diets containing canola meal (CM) and extruded soybean meal (ESBM)

Item	Treatment[1] CM	ESBM	SEM[2]	*P*-value[3]
EAA, µ*M*				
Arg	74.1	73.4	2.99	0.87
His	50.6	51.9	1.87	0.61
Ile	133.4	147.0	4.41	0.03
Leu	134.0	152.2	5.32	0.01
Lys	81.1	81.5	3.41	0.92
Met	25.4	24.6	1.12	0.60
Phe	39.8	44.4	1.24	0.01
Thr	95.3	90.8	3.55	0.33
Trp	38.9	37.1	1.24	0.32
Val	268.2	276.7	8.26	0.45
Total EAA	915.6	955.7	26.57	0.29
NEAA, µ*M*				
Ala	284.7	281.6	9.47	0.81
Asn	34.1	38.2	1.49	0.06
Asp	6.50	7.67	0.446	0.05
Cit	74.4	71.5	2.30	0.26
Cys	0.04	0.05	0.026	0.85
Gln	215.3	204.9	6.98	0.25
Glu	72.7	66.1	2.13	<0.001
Gly	308.8	289.9	15.08	0.26
Orn	52.6	49.8	2.27	0.39
Pro	83.0	85.3	3.09	0.54
Ser	83.5	87.1	3.46	0.47
Tau	57.7	52.1	2.79	0.16
Tyr	42.9	47.3	2.03	0.13
Total NEAA	1,317	1,280	38	0.46
Total EAA and NEAA	6,832	6,804	177	0.90
Carnosine	11.0	10.9	0.67	0.88
1-Methyl-L-histidine	14.0	17.3	0.69	<0.001
3-Methyl-L-histidine	4.05	4.00	0.17	0.86

[1]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[2]Largest SEM reported in table; n = 40 (n represents the number of observations used in the statistical analysis).

[3]Main effect of treatment.

and dLys. Duodenal flow of digestible EAA was greater for the ESBM diet when compared with the CM diet (1,366 vs. 1,188 g/d, respectively), likely resulting from greater RUP content and intestinal digestibility of ESBM relative to CM. Essential AA concentration in plasma only accumulate when supplied in excess of cow requirements (Almquist, 1954; Broderick et al., 1974). Typically, replacement of SSBM with CM in diets of lactating dairy cows results in increased plasma concentration of Met and Lys as a consequence of greater concentration of these AA in CM than SSBM (NRC, 2001). The meta-analysis by Martineau et al. (2014) also reported positive production effects of feeding CM, which was attributed to increased intestinal supply of digestible AA. In the report by Lage et al. (2021), cows fed the ESBM diet were deficient in dMet when compared with cows fed the CM diet, whereas Giallongo et al. (2015) reported increased plasma concentration of Met and His for cows fed ESBM relative SSBM, likely caused by increased RUP supply with ESBM. In the current experiment, the increased RUP supplied with the ESBM diet did not correspond to increased plasma concentration of Met, Lys and His. Despite

Table 7. Blood metabolites of cows fed diets containing canola meal (CM) and extruded soybean meal (ESBM)

Item	Treatment[1] CM	ESBM	SEM[2]	*P*-value[3]
Glucose, mg/dL	56.1	51.4	1.29	0.001
Urea N, mg/dL	24.3	23.9	0.99	0.70
BHB, µ*M*	681.5	661.3	40.0	0.67
Fatty acids, µ*M*	223.1	208.2	13.0	0.37

[1]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[2]Largest SEM reported in table; n = 40 (n represents the number of observations used in the statistical analysis).

[3]Main effect of treatment.

supplementation with RPLys, estimated duodenal flow of dLys was lower for cows fed the CM diet. Concentrations of BCAA such as Ile, Leu, and Phe were greater in ESBM when compared with CM. A greater dietary supply of EAA will lead to more EAA being absorbed at the small intestine and consequently increase plasma concentration of EAA (Broderick et al., 1974). Furthermore, it is known that EAA that are not required by the mammary gland return to peripheral circulation (Arriola Apelo et al., 2014). Thus, greater plasma concentrations of BCAA observed for cows fed the ESBM diet relative to CM likely reflects differences in AA supplied (vs. the demand) by the meals. These AA were also decreased in the SSBM and CM diets fed in the Lage et al. (2021) study, with no effects on milk true protein concentration. Increased plasma concentration of 1-MH has been reported for diets containing ESBM (extruded at 171°C) when compared with SSBM (Giallongo et al., 2015; Lage et al., 2021). Conversely, Harper et al. (2019) did not detect differences in plasma 1-MH when ESBM (extruded at 160°C) was added to the diet of early-lactation dairy cows in replacement of SSBM. In the Lage et al. (2021) study, there was no difference in plasma 1-MH between cows fed CM- and those fed ESBM-based diets. It should be noted that overall plasma 1-MH concentrations were higher in the current experiment when compared with values for ESBM-fed cows in Giallongo et al. (2015). As stated in Lage et al. (2021) and Giallongo et al. (2015), anserine is a precursor of 1-MH, but it is unclear how changes in anserine and consequently 1-MH in blood and urine occur in dairy cattle (Houweling et al., 2012). Plasma anserine concentrations were not detected in the current experiment.

Decreased plasma glucose concentration when cows were fed ESBM in replacement of SSBM has previously been reported (Broderick et al., 1990; Harper et al., 2019). However, replacement of SSBM with CM did not affect blood glucose concentration in the experiment by Maxin et al. (2013b). Rumen-derived propionate is known to be the principal gluconeogenic precursor in ruminants (Herbein et al., 1978). Therefore, the increased glucose concentration in plasma of cows fed the CM in the current experiment agrees with the increased molar proportion of propionate relative to cows fed the ESBM diet. In line with effects on milk composition of multiparous cows observed in the present study, increased blood glucose concentration of CM cows may have decreased the use of AA for gluconeogenesis and led to increased availability of AA for milk protein synthesis, compared with the ESBM diet (Lemosquet et al., 2004). The lack of effect of treatment on plasma FA and BHB concentrations in the current study is consistent with the lack of differences in production variables such as DMI, MY, BCS, and BW of cows.

Apparent Total-Tract Digestibility

Nutrient intake during experimental wk 7 (the digestibility sample collection week) and apparent total-tract digestibility data are shown in Table 8. Treatment did not affect DM and nutrient intakes. Digestibility of ADF was greater ($P = 0.02$) for ESBM relative to CM in the current experiment. Treatment did not affect total-tract digestibility of any other nutrients.

Results from previous studies investigating the effect of replacement of SSBM with ESBM on nutrient digestibility have been inconsistent. Harper et al. (2019) reported similar digestibility of all nutrients when replacing SSBM with ESBM extruded at 160°C, whereas Giallongo et al. (2015) observed increases in DM, OM, and ADF digestibilities when feeding ESBM extruded at 171°C relative to ESBM extruded at 149°C. On the other hand, decreased DM, OM, NDF and CP digestibilities have been reported for diets where CM replaced SSBM (Paula et al., 2018), whereas digestibilities of DM, OM and CP have been unaffected by CM substitution in other experiments (Brito and Broderick, 2007; Huhtanen et al., 2011). In the Lage et al. (2021) experiment, digestibility of DM, OM, NDF, and CP

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

Item	Treatment[1]			
	CM	ESBM	SEM[2]	P-value[3]
Intake,[4] kg/d				
DM	26.0	25.4	0.60	0.48
OM	24.4	24.0	0.56	0.53
NDF	9.02	8.66	0.210	0.21
ADF	5.06	4.98	0.117	0.59
CP	4.29	4.14	0.098	0.28
Starch	6.81	6.49	0.155	0.13
Apparent total-tract digestibility, %				
DM	69.9	69.6	0.38	0.46
OM	71.2	70.7	0.40	0.37
NDF	49.3	47.7	0.56	0.12
ADF	40.1	42.8	0.72	0.02
CP	73.5	72.3	0.61	0.15
Starch	98.4	98.2	0.15	0.21

[1]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[2]Largest SEM reported in table; n = 40 (n represents the number of observations used in the statistical analysis).

[3]Main effect of treatment.

[4]DMI reported is during the fecal and urine collection period (experimental wk 7) for the digestibility analysis.

were increased, whereas that of starch was decreased when either CM or ESBM replaced SSBM. In that experiment, apart from a greater CP digestibility for the ESBM diet, no differences were reported between CM and ESBM. In addition, these authors related the increased CP digestibility to the increased intestinal digestibility of ESBM relative to CM. The inverse relationship between DMI and passage rate and nutrient digestibility (Robertson and Van Soest, 1975) could also partially explain differences in digestibility in the Lage et al. (2021) experiment and lack thereof in the current one. Canola meal had lower intestinal RUP digestibility than ESBM in the current experiment, which, along with the higher RDP of CM, explains the similar total-tract CP digestibility and fecal N excretion (see following section) of the 2 diets. In a previous study from our group, increased DM and ADF digestibilities was reported when a high-temperature ESBM replaced a low-temperature ESBM (Giallongo et al., 2015). These results are in line with the greater digestibility of ADF in the ESBM diet, compared with CM, in the current experiment. The greater ADF digestibility observed for the ESBM diet in the current experiment could be attributed to: (1) addition of highly digestible fiber from soybean hulls in the ESBM diet that would stimulate fiber-degrading bacteria and fibrolysis in the rumen; (2) decreased ruminal availability of FA from ESBM, thus making ESBM FA less detrimental to ruminal bacteria (Chouinard et al., 1997a,b; Sterk et al., 2012); and (3) altered ruminal fermentation of fiber due to increased supply of UFA (Maia et al., 2007) in the form of canola oil in the CM diet. These processes, however, did not affect apparent total-tract digestibility of NDF.

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

Item	Treatment[1]			
	CM	ESBM	SEM[2]	P-value[3]
N intake, g/d[4]	686.5	663.2	15.74	0.28
N excretion or secretion, g/d				
Urine N	201.2	210.4	11.81	0.59
UUN[5]	111.2	132.9	10.68	0.16
Fecal N	195.9	184.4	6.79	0.22
Total excreta N	398.3	407.5	15.78	0.68
Milk N				
Primiparous	163.2	176.7	11.13	0.32
Multiparous	207.8	175.2	9.49	0.01
As % of N intake				
Urine N	29.7	31.9	1.77	0.39
UUN	16.4	20.1	1.51	0.09
Fecal N	28.6	27.8	0.69	0.45
Total excreta N	58.4	59.7	1.61	0.55
Milk N				
Primiparous	25.6	28.7	1.63	0.17
Multiparous	28.7	25.0	1.39	0.06
Unaccounted N	12.5	14.2	2.27	0.56
Urine output, kg/d	22.0	23.3	1.52	0.54
Urinary PD excretion, mmol/d				
Allantoin	596.5	625.8	38.34	0.58
Uric acid	58.9	66.6	4.60	0.20
Total PD	657.3	730.6	48.46	0.28

[1]Dietary treatments were CM diet (canola meal included at 15.8% of dietary DM) and ESBM diet (extruded soybean meal included at 13.2% of dietary DM).

[2]Largest SEM reported in table; n = 40 (n represents the number of observations used in the statistical analysis).

[3]Main effect of treatment. Treatment × parity interaction ($P \leq 0.03$) for milk N excretion (in g/d and as % of N intake).

[4]N intake reported is during the fecal and urine collection period (experimental wk 7).

[5]UUN = urinary urea nitrogen.

Nitrogen Utilization

Excretion of urinary and fecal N was not different between diets (Table 9). Urinary urea N excretion (as % of N intake), however, tended to be increased ($P = 0.09$) by the ESBM diet compared with CM. In multiparous cows only, secretion of milk N in grams/day was increased ($P = 0.04$), whereas secretion of milk N as % of N intake tended to be increased ($P = 0.06$) by the CM diet relative to ESBM. There was no effect of treatment on total excreta N or on excretion of urinary purine derivatives.

Secretion of milk N and milk true protein yield respond similarly to dietary CP (Olmos Colmentero and Broderick, 2006). Thus, the greater ($P = 0.02$) milk N secretion (in g/d, or as % of N intake, a trend at $P = 0.06$) in multiparous cows fed CM relative to ESBM, is related to the effect observed on milk protein yield and will not be further discussed in this section. When compared with CM, the greater RUP supply in multiparous cows fed the ESBM diet did not lead to greater milk N excretion likely as a result of already high MP supply provided by the diet and agrees with results in Lage et al. (2021).The greater UUN excretion with the ESBM diet than with CM is in agreement with the increased MUN and can be explained with the greater MP supply with the former diet that was not used for productive purposes.

CONCLUSIONS

In the conditions of the current experiment, RUP content of CM was greater than NRC (2001) or NASEM (2021) values and closer to RUP values for ESBM extruded at 163°C. Replacement of CM with ESBM in the diet of lactating cows did not affect DMI, MY, ECM, BW, and BW gain. Apart from experimental wk 5, ECM feed efficiency was similar between cows fed CM and ESBM diet. Cows fed ESBM had greater milk fat concentration and yield (by 13 and 11%, respectively) than cows fed the CM diet. In multiparous cows only, milk true protein yield was decreased for ESBM relative to CM. Compared with CM, ruminal concentration of total VFA, and the molar proportion of acetate were increased but propionate and valerate were decreased by ESBM. Overall, this experiment showed that substituting CM with ESBM, on an equal CP basis, in diets adequate in dMet and dLys and with similar EE and NDF contents, would result in similar lactational performance in dairy cows.

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