Effects of replacing soybean meal with canola meal differing in rumen-undegradable protein content on ruminal fermentation and gas production kinetics using 2 in vitro systems

E. M. Paula,* H. F. Monteiro,*† L. G. Silva,* P. D. B. Benedeti,*‡ J. L. P. Daniel,† T. Shenkoru,* G. A. Broderick,§ and A. P. Faciola*¹

*Department of Agriculture, Nutrition, and Veterinary Sciences, University of Nevada, Reno 89557

†Department of Animal Sciences, State University of Maringá, Maringá, Paraná, Brazil 87020-900

‡Department of Animal Sciences, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, 36570-000

§Broderick Nutrition and Research LLC, Madison, WI 53705

ABSTRACT

Previous research indicated that there were significant differences in rumen-undegradable protein (RUP) among canola meals (CM), which could influence the nutritional value of CM. The objectives of this study were to (1) evaluate the effects of feeding CM with different RUP contents on ruminal fermentation, nutrient digestion, and microbial growth using a dual-flow continuous culture system (experiment 1) and (2) evaluate ruminal gas production kinetics, in vitro organic matter (OM) digestibility, and methane (CH₄) production of soybean meal (SBM) and CM with low or high RUP in the diet or as a sole ingredient using a gas production system (experiments 2 and 3). In experiment 1, diets were randomly assigned to 6 fermentors in a replicated 3 × 3 Latin square. The only ingredient that differed among diets was the protein supplement. The treatments were (1) solvent-extracted SBM, (2) low-RUP solvent-extracted CM (38% RUP as a percentage of crude protein), and (3) high-RUP solvent-extracted CM (50% RUP). Diets were prepared as 3 concentrate mixtures that were combined with 25% orchardgrass hay and 15% wheat straw (dry matter basis). Experiments 2 and 3 had the same design with 24 bottles incubated 3 times for 48 h each. During the 48-h incubation, the cumulative pressure was recorded to determine gas production kinetics, in vitro OM digestibility, and CH₄ production. In experiment 1, N flow (g/d), efficiency of N use, efficiency of bacterial N synthesis, total volatile fatty acids (mM), and molar proportion of acetate, propionate, and isobutyrate were not affected by treatments. There were tendencies for a decrease in ruminal NH₃-N and an increase in molar proportion of butyrate for the SBM diet compared with both CM diets. The

molar proportion of valerate was greater in both CM diets, whereas the molar proportion of isovalerate and total branched-chain volatile fatty acids was lower for the CM diets compared with the SBM diet. In experiments 2 and 3, the SBM diet had a greater gas pool size than both CM diets. The SBM diet increased in vitro OM digestibility; however, it also tended to increase CH₄ production (mM and g/kg of DM) compared with both CM diets. Based on the results of this study, CM with RUP varying from 38 to 50% of crude protein does not affect ruminal fermentation, nutrient digestion, and microbial growth when CM is included at up to 34% of the diet.

Key words: ammonia nitrogen, rumen-degradable protein, rumen nitrogen metabolism, volatile fatty acid

INTRODUCTION

Canola meal (CM) and soybean meal (SBM) are common protein supplements fed to dairy cows in North America. Canadian solvent-extracted CM varies in nutritional composition because of environmental conditions during growth and harvest as well as cultivar variation and meal processing (Canola Meal Feeding Guide, 2015). We have shown in a survey including CM samples collected from 12 Canadian processing plants over 4 yr that CM varies in RUP content, ranging from 43 to 51% of its CP (Broderick et al., 2016), which may have an effect on ruminal N metabolism and postruminal N utilization.

Recent studies comparing CM with SBM have shown an increase in milk yield and milk protein yield and a decrease in MUN with CM as the major source of protein (Martineau et al., 2013). Furthermore, CM reduced ruminal ammonia nitrogen (NH₃-N) and branched-chain VFA (BCVFA) concentrations and decreased methane (CH₄) emissions per kilogram of ECM (Broderick et al., 2015; Gidlund et al., 2015). The reasons why CM improves milk and milk protein production,

Received November 13, 2016.

Accepted March 10, 2017.

¹Corresponding author: afaciola@cabnr.unr.edu

reduces MUN, and improves overall N utilization when replacing SBM are not clear. A better AA profile may play a role, but it is unclear whether a ruminal effect of feeding CM also plays a role in making CM a better protein supplement for dairy cows.

We know of no published studies reporting the effects of CM containing different RUP concentrations on rumen metabolism and gas production. Therefore, we selected from a subset of our previous survey study (Broderick et al., 2016) CM with the lowest (38%; **LCM**) and greatest (50%; **HCM**) RUP contents to assess whether this difference would affect ruminal N utilization. We also used a standard SBM source to serve as a control. We hypothesized that (1) CM with greater RUP content would increase RUP-N flow and decrease gas production compared with regular CM and (2) both CM diets would change ruminal fermentation, N metabolism, and total gas production compared with the SBM diet. Therefore, the objectives of this study were to evaluate the effects of feeding CM with different RUP concentration and SBM on (1) nutrient digestion, ruminal fermentation, N metabolism, and microbial protein synthesis using a dual-flow continuous culture system and (2) ruminal gas production kinetics, in vitro OM digestibility, and CH₄ production.

MATERIALS AND METHODS

Animal care and handling was approved by the University of Nevada, Reno Institutional Animal Care and Use Committee (protocol no. 00588).

Experiment 1

Diets and Experimental Design. Three diets were formulated to meet or exceed the dairy NRC recommendations (NRC, 2001) using as a reference a Holstein cow that produced 44 kg of milk, was 120 DIM, and had a BW of 660 kg. Diets were fed as TMR and contained (DM basis) 15% wheat straw, 25% orchard hay, and 60% concentrate. Dietary treatments were (1) SBM with 42.6% of RUP as a percentage of CP (NRC, 2001), (2) LCM (38% of RUP; Broderick et al., 2016), and (3) HCM (50% RUP; Broderick et al., 2016). Chemical composition of the diets is shown in Table 1. Chemical composition of the protein supplements is shown in Table 2. All ingredients were ground to pass through a 2-mm screen in a Wiley mill (model no. 2, Arthur H. Thomas Co., Philadelphia, PA). The diets were formulated to contain 18% CP; however, after feed analysis we found greater contents of CP for all ingredients used in the diets. Therefore, the final diet fed to the fermentors contained 21% of CP. Each fermentor was fed 72 g/d of DM equally divided into 4 meals at 0600, 1200,

Table 1. Ingredient and chemical composition of diets (% of DM unless otherwise stated)

Item	Diet ¹		
	SBM	LCM	HCM
Orchard hay	25.0	25.0	25.0
Wheat straw	15.0	15.0	15.0
Ground corn	32.5	24.3	23.0
SBM	25.0	—	—
LCM	—	33.2	—
HCM	—	—	34.5
Vitamin and mineral premix	2.5	2.5	2.5
Chemical composition			
OM	91.5	90.9	90.6
CP	21.5	20.7	20.6
RDP ²	14.5	13.2	12.9
RUP ²	7.0	7.5	7.7
RDP ³	13.3	13.1	12.6
RUP ⁴	8.2	7.7	8.1
NDF	31.1	37.8	37.5
ADF	18.2	24.5	25.2
NFC ⁵	41.3	36.0	36.3
Ether extract	2.22	2.15	1.82
NDIN, % of total N	7.7	15.9	14.8
NE _L , ⁶ Mcal/kg of DM	1.58	1.50	1.50

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Estimated using the NRC (2001) model.

³Calculated from in vitro dual-flow continuous culture measurements as RDP (% of DM) = [total CP intake (g/d) – effluent RUP flow (g/d)] × 100/DMI (g/d). Adapted from Reynal and Broderick (2005).

⁴Calculated from in vitro dual-flow continuous culture measurements as RUP (% of DM) = [total effluent CP flow (g/d) – effluent bacterial CP flow (g/d)] × 100/DMI (g/d). Adapted from Reynal and Broderick (2005).

⁵NFC = 100 – (% NDF + % CP + % fat + % ash) + % NIDN × 6.25 according to the NRC (2001) model.

⁶Estimated using the NRC (2001) model.

1800, and 2400 h. Diets were randomly assigned within squares to 6 dual-flow continuous culture fermentors in a replicated 3 × 3 Latin square arrangement with three 10-d experimental periods consisting of 7 d for diet adaptation and 3 d for sample collection.

Dual-Flow Continuous Culture System. For this study, 6-unit dual-flow continuous culture fermentors (Omni-Culture Plus; Virtis Co. Inc., Gardiner, NY) originally developed by Hoover et al. (1976) and recently modified by Benedetti et al. (2015) and Silva et al. (2016) were used. Ruminal fluid was collected approximately 2 h after feeding from 2 rumen-cannulated steers (average BW = 550 kg). The donor steers were fed (DM basis) a 60:40 forage:concentrate diet containing 60% grass hay, 27.5% ground corn, 10% SBM, and 2.5% vitamin premix. Ruminal digesta was manually collected and strained through 4 layers of cheesecloth, and approximately 10 L of ruminal fluid was poured into a prewarmed insulated vessel. The rumen fluid was pooled and homogenized, infused with N₂ to maintain

Table 2. Chemical composition of the protein supplements (% of DM unless otherwise stated)

Chemical composition	Protein supplement ¹		
	SBM	38% RUP CM	50% RUP CM
OM	92.7	92.5	91.8
CP	54.9	43.6	42.1
RDP	31.5 ²	26.9 ³	21.2 ³
RUP	23.4 ²	16.7 ³	20.9 ³
NDF	10.3	30.4	28.7
ADF	6.2	23.0	24.2
NFC ⁴	27.1	18.1	18.0
Ether extract	1.1	1.66	0.81
NDIN, % of total N	0.5	25.3	21.3
ME, ⁵ Mcal/kg of DM	3.41	2.75	2.75

¹SBM = standard soybean meal; 38% RUP CM = canola meal with 38% RUP; 50% RUP CM = canola meal with 50% RUP.

²Estimated using the NRC (2001) model for a cow with DMI = 4% of BW.

³Estimated according to Broderick et al. (2016).

⁴NFC = 100 – (% NDF + % CP + % fat + % ash) + NDIN × 6.25 according to NRC (2001).

⁵Estimated using the NRC (2001) model.

the anaerobic environment, and kept at 39°C in a 5,000-mL Erlenmeyer flask in a preheated water bath. The rumen fluid was poured into each of the prewarmed fermentors until it cleared the effluent spout.

Fermenter contents were continuously stirred by a central propeller apparatus driven by magnets at the rate of 150 rpm. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused at 2.2 mL/min. Liquid and solid dilution rates were adjusted daily to 11 and 5.5%/h, respectively, by adjusting buffer input and liquid and solid removal. Individual pH controllers (Cole-Parmer model 5997–20; Vernon Hills, IL) were used to monitor the pH of each fermentor.

On d 5, effluent digesta (liquid and solid) were homogenized and samples collected to determine the background ¹⁵N abundance. Then, 0.077 g of (¹⁵NH₄)₂SO₄ with 10.2% atom excess of ¹⁵N (Sigma-Aldrich Co., St. Louis, MO) was infused into each fermentor to instantaneously label the NH₃-N pool. Saliva was reformulated to contain 0.077 g/L of the enriched (¹⁵NH₄)₂SO₄ to replace an isonitrogenous amount of urea to obtain a steady-state ¹⁵N enrichment of the NH₃ pool in the fermentors (Calsamiglia et al., 1996).

Liquid and solid effluents were collected in 4.3-L plastic containers. During the first 7 d (adaptation period), the effluent containers were weighed once daily at 0600 h and the contents were discarded. Twenty-four hours before the first collection and during the 3-d sampling period, liquid and solid effluent containers were immersed in a chilled water bath at 2°C, and 20 mL of 50% H₂SO₄ was added to each container to prevent further microbial and enzymatic activities.

On d 8, 9, and 10, liquid and solid digesta effluents from each fermentor were taken and homogenized for

1 min (T25 basics, IKA Works Inc., Wilmington, NC), and 500 mL was removed via a vacuum system and stored at –20°C for later analysis of DM, OM, CP, NDF, and ADF. Additionally, two 10-mL subsamples were filtered through 8 layers of cheesecloth, preserved with 0.2 mL of 0.2 N sulfuric acid, and centrifuged at 1,000 × *g* for 15 min at 4°C. The supernatant was decanted and stored at –20°C for subsequent ruminal NH₃-N and VFA analysis. Fermenter pH was measured with an Accumet portable AP61 pH meter (Fisher Scientific, Atlanta, GA) at 0600, 0800, 0900, 1000, and 1100 h.

On d 10, the entire fermentor contents were strained through 2 layers of cheesecloth and centrifuged at 1,000 × *g* for 10 min at 5°C. Then, the supernatant was centrifuged (Sorvall RC-5B refrigerated superspeed centrifuge; DuPont, Wilmington, DE) at 20,000 × *g* for 20 min at 5°C. The supernatant was discarded, and bacterial pellets were freeze dried and stored for further analysis of ¹⁵N, N, and OM (Bach et al., 2008).

Chemical Analyses. Feed and effluent samples were analyzed for DM (method 934.01), ether extract (method 920.85), and ash (method 938.08) according to AOAC (1990). The CP content of feed samples was determined using a Leco combustion nitrogen analyzer (Leco CN628 Carbon/Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI; method 990.13, AOAC International, 2005). The OM content was calculated as the difference between DM and ash contents. For NDF, samples were analyzed, treated with thermostable α-amylase and sodium sulfite according to Mertens (2002), and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY). For ADF, samples were sequentially analyzed according to Van Soest and

McQueen (1973) and adapted for the Ankom200 Fiber Analyzer. Neutral detergent-insoluble CP (NDICP) was isolated by gravimetric determination using thermostable α -amylase and sodium sulfite followed by CP analysis (method 990.13; AOAC International, 2005). The NFC concentration of the feed ingredients was calculated using the equation $\text{NFC} = 100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ ash}) + \text{NDICP}$ (according to NRC, 2001).

Concentration of VFA of the effluent samples was determined with a gas chromatograph (model 3800; Varian Inc., Walnut Creek, CA) equipped with a glass column (180 cm $\times$ 4 mm i.d.) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW (Supelco, Bellefonte, PA); N_2 was used as a carrier gas at a flow rate of 85 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180°C, respectively. Ruminal NH_3 -N concentration was determined according to Chaney and Marbach (1962). Bacterial and effluent digesta samples were analyzed for total N as previously described. Bacterial, effluent digesta, and background samples were analyzed for enrichment with ^{15}N using a Eurovector model 3000 elemental analyzer (Euro EA 3000, Eurovector S.P.A., Milano, Italy) interfaced to a Micromass Isoprime stable isotope ratio mass spectrometer (IsoPrime, Micromass UK Ltd., Manchester, UK; Werner et al., 1999).

Calculations. Bacterial N flow and bacterial efficiency were calculated according to Calsamiglia et al. (1996) as follows:

$$\text{sample } ^{15}\text{N enrichment (atom percentage excess)} = \frac{\text{sample } ^{15}\text{N atom \%} - \text{background } ^{15}\text{N atom \%}}{\text{sample } ^{15}\text{N atom \%} - \text{background } ^{15}\text{N atom \%}},$$

$$\text{bacterial N flow (g/d)} = \frac{(\text{NAN flow} \times \text{atom percentage excess of } ^{15}\text{N of effluent})}{(\text{atom percentage excess of } ^{15}\text{N of bacteria}), \text{ and}}$$

$$\text{bacterial efficiency} = \frac{\text{bacterial N flow (g)}}{\text{OM truly digested (kg)}}.$$

True ruminal (DM, OM, CP, NDF, ADF) digestibilities were calculated as follows (using DM as an example):

$$\text{true DM digestibility (\%)} = \frac{[\text{g of dietary DM} - (\text{g of effluent DM} - \text{g of saliva DM} - \text{g of bacterial DM})]}{(\text{g of dietary DM})} \times 100.$$

Nitrogen flows were calculated as follows:

$$\text{NH}_3\text{-N flow (g/d)} = \frac{\text{mg/dL of effluent NH}_3\text{-N}}{(\text{g of total effluent flow/100})},$$

$$\text{NAN flow (g/d)} = \text{g of effluent N} - \text{g of effluent NH}_3\text{-N},$$

$$\text{dietary N flow (g/d)} = \text{g of effluent NAN} - \text{g of effluent bacterial N},$$

$$\text{RUP-N flow (g/d)} = \text{total N flow} - \text{effluent bacterial N flow, and}$$

$$\text{RDP-N supply (g/d)} = \text{total N intake} - \text{RUP-N flow}.$$

Experiment 2

Experiment 2 aimed to evaluate ruminal gas production kinetics, in vitro OM digestibility, and CH_4 and ATP production from the 3 diets used in experiment 1. A gas production apparatus (Ankom RF gas production system, Ankom Technology) equipped with pressure sensors that were wirelessly connected to a computer was used. The experimental design was 3 incubation runs $\times$ 3 diets $\times$ 6 bottles per treatment plus 18 blank bottles (6 per run), totaling 72 units. Dietary treatments were similar to those used in experiment 1 (Table 1) with the exception that the mineral premix was not used. The mineral premix used in experiment 1 was not used in experiments 2 and 3 because in experiments 2 and 3 a buffer-mineral solution was used as recommended by Tagliapietra et al. (2011) and Menke and Steingass (1988), providing all minerals needed for microbial growth.

Feed ingredients were ground to pass through a 1-mm screen in a Wiley mill (model no. 2; Arthur H. Thomas Co.). Each bottle (620 mL) was filled with 0.5 g of each diet. Samples were hydrated with deionized water to avoid particle dispersion. The buffer mineral solution was prepared according to Menke and Steingass (1988) except for the addition of sodium sulfite and L-cysteine. The buffer mineral solution was kept in a water bath at 39°C and purged continuously with N_2 infusion for 30 min. The resazurin solution was used as a color indicator for monitoring buffer pH and N_2 saturation (oxidation-reduction potential). Rumen fluid was collected from the same animals used in experiment 1, which were on the same diet described previously. Two hours after feeding, 2 L of rumen fluid was collected, immediately filtered through 4 layers of cheesecloth, and held in prewarmed thermal containers (39°C). The rumen fluid was mixed with the buffer

solution (1:2 vol/vol) in a water bath at 39°C under anaerobic conditions by flushing with N₂ (Menke and Steingass, 1988). Bottles were inoculated with 75 mL of rumen fluid–buffer mixture solution while the bottle headspace was continuously flushed with N₂. Bottles without feed samples but with the rumen fluid–buffer mixture solution were used as blanks to correct for rumen inoculum fermentation. After inoculation, bottles were closed and placed in an air-ventilated shaker incubator (Innova 4400 incubator shaker; New Brunswick Scientific, Edison, NJ) under controlled temperature and agitation (39°C and 80 rpm). The data acquisition software (Gas Pressure Monitor, Ankom Technology) was set to record cumulative pressure every 15 min for 48 h. Valves were set to automatically release the gas when the pressure reached 3.4 kPa (Tagliapietra et al., 2011). At the beginning (0 h) and end (48 h) of the incubation, the solution pH was measured with an Accumet portable AP61 pH meter (Fisher Scientific).

At the end of the incubation, 10-mL subsamples were filtered through 2 layers of cheesecloth from half of the bottles (9 observations per treatment), and 0.2 mL of a 50% H₂SO₄ solution was added for later VFA determination and CH₄ production calculation. All the remaining bottles (9 observations per treatment) were stored and freeze dried for later determinations of DM and NDF.

Experiment 3

Experimental Procedures and Substrates. Experiment 3 evaluated ruminal gas production kinetics, in vitro OM digestibility, and CH₄ and ATP production from the 3 protein supplements as a sole ingredient. This experiment used the same gas production apparatus system, experimental design, and 3 protein supplements used in experiments 1 and 2. The chemical composition of the ingredients is shown in Table 2. The 3 protein supplements were also ground to pass through a 1-mm screen in a Wiley mill (model no. 2; Arthur H. Thomas Co.). As described earlier for experiment 2, at the end of the incubation, half of the bottles were used for VFA sampling and the remaining bottles were stored for later DM and NDF determination.

Chemical Analysis and Calculations (Experiments 2 and 3). The 3 protein supplements and the ingredients used in the experimental diets were analyzed for DM, OM, CP, NDF, ADF, NDIN, and NDF as described in experiment 1. The VFA concentrations were determined using gas chromatography as described in experiment 1. Postfermentation freeze-dried samples were analyzed for DM and subsequently for NDF.

Calculations (Experiments 2 and 3). The ATP and CH₄ production was estimated according to Owens

and Goetsch (1988) through VFA stoichiometry using the following equations:

$$\text{ATP} = (1/4 \text{ acetate}) + (2 \text{ } 3/4 \text{ propionate}) \\ + (3 \text{ } 1/2 \text{ butyrate}),$$

$$\text{CH}_4 = (1/2 \text{ acetate}) + (1/2 \text{ butyrate}) \\ - (1/4 \text{ propionate}).$$

The in vitro true OM digestibility (%) was calculated as (iOM – rNDF)/iOM, where iOM is the incubated OM and rNDF is the residual NDF after 48 h of digestion minus the NDF content in the blank bottles.

For cumulative pressure (kPa), the gas pressure was converted to units of volume according to Tagliapietra et al. (2011) that used the ideal gas law in which gas pressure (mL) = (Pc/Po) × Vo, where Pc is the cumulated pressure change (kPa) in the bottle headspace, Vo is the bottle headspace volume (545 mL), and Po is the atmospheric pressure read by the equipment at the beginning of the measurement. The in vitro digestibility and the final gas production volumes were corrected by subtracting the blank bottle values.

Statistical Analysis

Experiment 1. Data were analyzed using the MIXED procedure of SAS 9.4 (SAS, 2002) as a replicated 3 × 3 Latin square design according to the following model:

$$Y_{ijkl} = \mu + S_i + P_j + F(S)_{ki} + D_l + E_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of Latin square ($i = 1$ or 2), P_j is the fixed effect of period ($j = 1$ – 3), $F(S)_{ki}$ is the random effect of fermentor (F) within square ($k = 1$ – 6), D_l is the fixed effect of diet ($l = \text{SBM, LCM, or HCM}$), and E_{ijkl} is the residual error. Orthogonal contrasts were constructed to evaluate the effect of the different protein supplements as follows: (1) SBM versus LCM + HCM and (2) LCM versus HCM. Least squares means and standard error of the mean are reported for all data, with significance declared at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$. Ruminal pH data were analyzed as repeated measures according to the following model:

$$Y_{ijklm} = \mu + S_i + P_j + F(S)_{ki} + D_l + T_m \\ + DT_{lm} + E_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, S_i is the fixed effect of Latin square ($i = 1$ or

2), P_j is the fixed effect of period ($j = 1-3$), $F(S)_{ki}$ is the random effect of fermentor (F) within square ($k = 1-6$), D_l is the fixed effect of diet ($l = \text{SBM, LCM, or HCM}$), T_m is the fixed effect of time ($m = 1-5$), DT_{lm} is the interaction between diet and time, and E_{ijklm} is the residual error. The effect of fermentor within diet was used as the error term to test the effect of diet. The covariance structure used was the one with the smaller value for the Akaike information criterion.

Experiments 2 and 3. Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, 2002) with a statistical model including fixed effect of treatment and random effect of run. Orthogonal contrasts were as described for experiment 1. An exponential model with lag phase was fitted to the cumulative gas production using the NLIN procedure of SAS 9.4 (SAS Institute, 2002) to analyze ruminal gas production kinetics over 48 h in experiments 2 and 3. Least squares means and standard error of the mean are reported for all data. Significance was declared at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Ruminal pH and N Metabolism. No differences in rumen pH were found across diets. The average for all dietary treatments was 6.4; a minor variation was found between feed intervals (minimum = 6.4; maximum = 6.6). Bach et al. (2005) noted that ruminal pH lower than 5.5 could decrease protein degradation;

therefore, in the present study, pH likely did not affect protein degradation.

Diet had no effect ($P > 0.05$) on ammonia N concentration, total N, NAN, bacterial N, dietary N, RUP-N flows, RDP-N supply, efficiency of N utilization, and efficiency of bacterial N synthesis (Table 3). Although there was a trend ($P = 0.09$) for lower $\text{NH}_3\text{-N}$ flow when SBM was fed, the values observed were too similar to reflect in a meaningful biological response. It is important to highlight that in the present study all treatments were adjusted to receive the same amount of feed (72 g/d on a DM basis) and to have the same ruminal passage rate. Therefore, we were able to minimize the possible effects of DMI and passage rate on N metabolism. Two meta-analysis studies (Huhtanen et al., 2011; Martineau et al., 2013) reported an increase in DMI for diets supplemented with CM compared with SBM. Huhtanen et al. (2011) suggested that CM diets provide a better AA supply and support greater milk yield, which increases energy demand and consequently increases DMI. Therefore, because in our study we did not find any significant difference in N metabolism among the CM and SBM diets, it is possible that the positive effects of CM on N metabolism and milk production are correlated with the increase in DMI and a potentially better AA supply for CM diets compared with SBM diets.

Although the high dietary CP might have played a role in ruminal N fermentation, especially with regards to RDP and $\text{NH}_3\text{-N}$ levels, previous studies with 16.5 and 15.7% CP, respectively, reported similar results with regards to ruminal N metabolism when comparing

Table 3. Effects of different protein supplements on pH and N metabolism in dual-flow continuous culture (experiment 1)

Item	Treatment ¹				P-value ²	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
pH	6.40	6.50	6.43	0.07	0.10	0.11
$\text{NH}_3\text{-N}$, mg/dL	15.7	17.6	16.3	0.67	0.12	0.17
N flows, g/d						
Total N	2.51	2.41	2.44	0.05	0.24	0.67
$\text{NH}_3\text{-N}$	0.51	0.56	0.53	0.02	0.09	0.19
NAN	2.00	1.84	1.91	0.06	0.15	0.45
Bacterial N	1.56	1.53	1.51	0.04	0.38	0.81
Dietary N	0.44	0.31	0.40	0.05	0.23	0.28
RUP-N	0.94	0.88	0.93	0.05	0.57	0.54
RDP-N supply	2.07	2.04	1.98	0.07	0.44	0.47
ENU, ³ %	76.0	74.8	76.3	2.27	0.86	0.66
Bacterial efficiency ⁴	46.5	45.7	46.7	2.45	0.90	0.77

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Orthogonal contrasts for effects of different protein supplements (SBM vs. LCM + HCM and LCM vs. HCM).

³Efficiency of N use = g of bacterial N/g of available N (Bach and Stern, 1999).

⁴Bacterial efficiency = g of bacterial N/kg of OM truly digested.

SBM and CM (Brito and Broderick, 2007; Paula et al., 2016a). This suggests that dietary CP levels play a minor role when comparing these 2 protein supplements. Lower CP levels would have made it challenging to evaluate RUP flow because low CP levels would leave little room for RUP differences among treatments. For example, a 16% dietary CP would yield a difference of only 19.2 g of RUP/kg of DMI between the 2 CM diets tested (38 and 50% RUP as a percentage of CP) as opposed to 25.2 g of RUP/kg of DMI in a 21% dietary CP level. Nevertheless, high CP levels may have precluded better evaluation of ruminal N fermentation.

We observed that the values for NAN, bacterial N, dietary N flows, and RDP-N supply were similar among the diets, indicating that ruminal CP degradability is similar between the CM and SBM diets. However, most feed tables (AFRC, 1993; NRC, 2001; INRA, 2007) report greater MP for SBM compared with CM because of its lower CP degradability and greater RUP content (Huhtanen et al., 2011). It is possible that feed tables have inaccurate CM RUP and RDP values, which could be attributable to methodological assessments of RDP and RUP. For example, early studies reporting CM RDP and RUP fraction used *in situ* methodology, which assumes that soluble proteins, peptides, and AA are completely degraded in the rumen, which may not always be the case (Reynal et al., 2007). Furthermore, Hedqvist and Udén (2006) have shown that the ruminal degradation rate of the soluble protein fraction of CM is lower than previously thought. In addition, Bach et al. (2008) found that 63% of the soluble CP fraction in CM may escape from rumen degradation. Also, *in situ* methodologies may impose physical restrictions to feed within the bags and contain microbial contamination in undigested residues (Broderick et al., 1991). Another possible reason for the discrepancy in CM RUP and RDP values in feed tables may be differences in seed processing or crushing methods. For example, NRC (2001) lists only mechanically extracted CM (expeller CM); however, solvent extraction of the CM is the primary method that is currently being used.

Maxin et al. (2013), evaluating *in situ* CP ruminal degradation of SBM and CM, observed lower CP degradability and greater RUP content for CM compared with SBM. Broderick et al. (2015) observed a significant decrease in ruminal $\text{NH}_3\text{-N}$ concentration for CM compared with SBM in lactating dairy cows. Furthermore, Brito et al. (2007) estimated numerical *in vivo* RUP flows of 29 and 34% (of CP) for SBM and CM diets, respectively. Therefore, our results are in agreement with previous results in the literature indicating that MP, RDP, and RUP content of CM should be revised in the feed tables to more accurately reflect the MP of CM in feed protein evaluation systems.

We did not observe differences in RUP flow, which was surprising given the magnitude of RUP differences between the CM sources that were used. For example, considering an average of 21% dietary CP, for the LCM diet 79.8 g of RUP was consumed for each 1 kg of DMI, whereas for the HCM diet 105 g of RUP was consumed for each 1 kg of DMI. The difference may not have been enough to affect ruminal N metabolism. Another possible explanation would be that the RUP values measured in the CM as a single ingredient (Broderick et al., 2016) may be slightly different when TMR were fed, meaning that possible interactions among ingredients may affect protein degradation.

VFA. Dietary treatments did not affect total VFA concentration; molar proportions of acetate, propionate, and isobutyrate; and acetate:propionate ratio (Table 4). These results are in agreement with other studies comparing SBM with CM, in which significant differences for total VFA and VFA molar proportions were not found (Sanchez and Claypool, 1983; Brito and Broderick, 2007). However, there were trends for increasing total VFA concentration ($P = 0.08$) and molar proportion of butyrate ($P = 0.06$) for the SBM diet compared with both CM diets. This might be attributable to greater NFC content in the SBM diet compared with the CM diets (41.3, 36.0, and 36.3%, respectively), which could contribute to more microbial fermentation compared with the CM diets. Testing SBM and CM as protein supplements, Brito and Broderick (2007) had diets differing in NFC content at approximately 4 percentage units (50.2 and 46.4% for SBM and CM, respectively) and did not find differences in total VFA and molar proportions of acetate and propionate. Moreover, also comparing SBM and CM, Broderick et al. (2015) had diets differing in NFC content at approximately 3 percentage units (47.8 and 44.5% for SBM and CM, respectively) and did not find differences in total VFA and molar proportions of acetate and propionate. Therefore, despite the NFC differences in the current study, it is unlikely that this difference played a major role in ruminal fermentation.

The molar proportion of valerate was greater ($P = 0.01$) when CM was fed, whereas molar proportions of isovalerate ($P = 0.02$) and total BCVFA were lower ($P = 0.04$) for CM diets. Ruminal BCVFA are products of branched-chain AA (valine, isoleucine, and leucine) oxidative deamination and decarboxylation (Allison and Bryant, 1963). Therefore, these BCVFA are associated with degradation of the branched-chain AA leucine, isoleucine, and valine, suggesting less degradation of these branched-chain AA for CM diets. In agreement with our findings, Broderick et al. (2015) reported a decrease in ruminal isovalerate and total BCVFA when CM replaced SBM in diets fed to lactating dairy cows.

Table 4. Effects of different protein supplements on VFA concentration in dual-flow continuous culture (experiment 1)

Item	Treatment ¹				P-value ²	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
Total VFA, mM	122.6	116.2	116.6	2.54	0.08	0.90
VFA, % of total VFA						
Acetate	64.6	65.4	66.5	1.25	0.26	0.42
Propionate	19.9	20.7	21.1	0.86	0.20	0.59
Butyrate	11.7	10.9	9.41	0.64	0.06	0.11
Isobutyrate	0.44	0.48	0.44	0.02	0.60	0.26
Valerate	1.51	1.63	1.63	0.03	0.01	0.97
Isovalerate	1.27	1.08	1.10	0.08	0.02	0.75
Acetate:propionate	3.26	3.20	3.18	0.18	0.63	0.94
Total BCVFA, ³ mM	2.08	1.77	1.76	0.11	0.04	0.67

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Orthogonal contrasts for effects of different protein supplements (SBM vs. LCM + HCM and LCM vs. HCM).

³BCVFA = branched-chain VFA.

Ruminal True Digestibility. Ruminal true digestibility was not affected by dietary treatments (Table 5). The average values for true DM, OM, NDF, and ADF ruminal digestibility were 43.3, 52.0, 74.8, and 66.4%, respectively. Because the main focus was on protein supplements, diets were relatively similar. Therefore, it was not expected that there would be major differences in DM, OM, and fiber digestibilities. Brito et al. (2007) evaluated the effect of different protein supplements on omasal nutrient flow in lactating dairy cows and did not observe significant differences for ruminal degradability of DM, OM, NDF, and ADF between the SBM and CM treatments, which agrees with our findings. In a review and meta-analysis evaluating CM as a protein supplement for dairy cows, Huhtanen et al. (2011) did observe reduced total-tract true CP digestibility for heat-treated CM diets compared with untreated CM diets. In the present study, there were no significant differences in ruminal CP digestibility between LCM and HCM diets. It is important to highlight that herein we measured ruminal CP digestibility only as opposed to total-tract CP digestibility. Similar to our results,

Ahvenjärvi et al. (1999) did not observe differences in ruminal CP digestibility between rapeseed meal and heat-moisture-treated rapeseed cake for lactating dairy cows. In addition, in another study we did not observe a difference in ruminal CP digestibility between regular CM and heat-treated CM; however, we did observe a tendency for a decrease in apparent total-tract CP digestibility for the heat-treated CM diet compared with the regular CM diet (Paula et al., 2016b). Therefore, it is likely that the lack of effects on ruminal CP digestibility between CM diets may be attributable to the small difference in RUP content between the diets.

Experiment 2

Means of gas production profiles of the diets during 48 h of fermentation are presented in Figure 1, and least squares means are presented in Table 6. As rumen fluid was collected 2 h after the morning feeding, it was assumed that the ruminal microorganisms were in the exponential phase of growth when rumen fluid was used for incubation. For that reason, the lag phase was

Table 5. Effects of different protein supplements on true ruminal nutrient digestibility in dual-flow continuous culture (experiment 1)

Ruminal true digestibility, %	Treatment ¹				P-value ²	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
DM	44.8	42.1	42.5	3.55	0.57	0.92
OM	52.3	53.0	50.7	3.97	0.90	0.57
CP	83.5	85.6	83.5	3.23	0.72	0.53
NDF	74.1	76.0	74.5	3.78	0.80	0.78
ADF	66.8	65.2	67.2	3.76	0.89	0.69

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Orthogonal contrasts for effects of different protein supplements (SBM vs. LCM + HCM and LCM vs. HCM).

Figure 1. Ruminal gas production profiles of the diets used in experiments 1 and 2 determined with the Ankom gas production system (Ankom Technology, Macedon, NY). SBM = dietary treatment with solvent soybean meal as a protein supplement; LCM = dietary treatment with 38% RUP canola meal as a protein supplement; HCM = dietary treatment with 50% RUP canola meal as a protein supplement.

considered as apparent (Pirt, 1975). Although diets did not differ in rate of gas production (0.06 mL/h average for all treatments; $P = 0.56$), the SBM diet had a

greater gas pool size (potentially fermentable fraction) than did both CM diets (342 mL/g of DM vs. 306 and 309 mL/g of DM for SBM vs. LCM and HCM diets, respectively; $P < 0.01$). However, LCM and HCM diets did not differ from each other ($P > 0.05$).

The greater gas pool size for the SBM diet may have been attributable to a greater OM digestibility after 48 h compared with both CM diets (Table 6). According to Ramin and Huhtanen (2013), an increase in CH_4 production (mol/d) is positively correlated with diet digestibility. Another reason that could explain the greater gas pool for the SBM diet may be a greater BCVFA concentration in the SBM diet (data not shown), which may have contributed to the growth of cellulolytic and some noncellulolytic bacteria (Allison, 1969).

Dietary treatments changed total VFA concentration and acetate and butyrate molar proportions (Table 6). Total VFA concentration increased in the SBM diet compared with the CM diets, which may be explained by the greater in vitro OM digestibility observed in the SBM diet. There was an increase in butyrate and a decrease in acetate molar proportions in the SBM diet compared with the CM diets. These changes may be attributable to the conversion of acetate into butyrate, which may occur when diets high in soluble carbohydrates are fed (Demeyer, 1991). Similar results were observed in experiment 3 (Table 7).

Table 6. Effects of different protein supplements on in vitro gas production parameters and digestibility (experiment 2)

Item	Treatment ¹				P-value ²	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
Final pH	6.92	7.03	7.21	0.02	<0.01	<0.01
Degradability kinetics						
Lag phase, h	0.83	0.65	0.68	0.17	0.14	0.78
Fermentation rate, mL/h	0.06	0.06	0.06	0.002	0.56	0.12
Gas pool size, mL/g of DM	342	306	309	12.00	<0.01	0.61
Total GP ₂₄ , ³ mL/g of DM	260	248	225	9.30	<0.01	<0.01
Total GP ₄₈ , ³ mL/g of DM	309	312	276	10.40	<0.01	<0.01
iv-tOMd, ⁴ %	75.0	71.5	69.9	1.90	<0.01	0.11
OMd _{GP24} , ⁵ %	83.5	84.1	77.7	1.71	<0.01	<0.01
Total VFA, mM	41.5	37.6	36.6	2.99	0.05	0.70
Acetate, % of total VFA	64.3	66.4	65.1	0.68	<0.01	0.02
Propionate, % of total VFA	18.1	18.8	17.8	0.96	0.79	0.24
Butyrate, % of total VFA	13.3	10.6	12.8	1.42	0.04	0.02
ATP ⁶ mM	46.6	39.7	40.2	3.35	<0.01	0.84
CH ₄ , ⁶ mM	14.2	12.7	12.6	0.98	0.07	0.93
CH ₄ , g/kg of digestible OM	40.8	41.5	40.1	5.26	0.99	0.63
CH ₄ , g/kg of DM	34.1	30.5	30.3	2.33	0.07	0.93

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Orthogonal contrasts for effects of different protein supplements (SBM vs. LCM + HCM and LCM vs. HCM).

³Total gas produced after 24 and 48 h of incubation for each gram of DM incubated.

⁴In vitro true OM digestibility estimated after 48 h of digestion.

⁵OM gas production digestibility estimated through cumulative gas production at 24 h (equation 43f in Menke and Steingass, 1988).

⁶Estimated through VFA production (Owens and Goetsch, 1988).

Table 7. Effects of different protein supplements on in vitro gas production parameters and digestibility (experiment 3)

Item	Treatment ¹				P-value ²	
	SBM	LCM	HCM	SEM	SBM vs. LCM + HCM	LCM vs. HCM
Final pH	7.28	7.29	7.28	0.06	0.51	0.54
Degradability kinetics						
Lag phase, h	0.44	0.27	0.08	0.11	<0.01	0.01
Fermentation rate, mL/h	0.11	0.10	0.09	0.01	<0.01	<0.01
Gas pool size, mL/g of DM	281	239	238	8.6	<0.01	0.96
Total GP ₂₄ , ³ mL/g of DM	257	212	198	9.5	<0.01	0.13
Total GP ₄₈ , ³ mL/g of DM	284	240	233	10.3	<0.01	0.49
iv-tOMd, ⁴ %	98.3	80.1	81.4	1.07	<0.01	0.31
OMd _{GP24} , ⁵ %	88.9	76.6	73.5	1.56	<0.01	0.05
Total VFA, mM	48.7	39.0	36.2	1.16	<0.01	0.10
Acetate, % of total VFA	63.5	64.7	65.3	1.03	<0.01	0.09
Propionate, % of total VFA	19.1	19.6	19.7	1.27	0.06	0.73
Butyrate, % of total VFA	10.5	9.41	8.99	1.01	<0.01	0.13
ATP, ⁶ mM	51.5	40.3	37.0	1.62	<0.01	0.07
CH ₄ , ⁶ mM	15.7	12.6	11.7	0.56	<0.01	0.19
CH ₄ , g/kg of digestible OM	41.6	40.9	38.2	1.88	0.30	0.18
CH ₄ , g/kg of DM	37.5	30.3	28.3	1.38	<0.01	0.18

¹SBM = solvent-extracted soybean meal; LCM = low-RUP solvent-extracted canola meal (38% RUP as a percentage of CP); HCM = high-RUP solvent-extracted canola meal (50% RUP).

²Orthogonal contrasts for effects of different protein supplements (SBM vs. 38% RUP CM + 50% RUP CM and 38% RUP CM vs. 50% RUP CM).

³Total gas produced after 24 and 48 h of incubation for each gram of DM incubated.

⁴In vitro true OM digestibility estimated after 48 h of digestion.

⁵OM gas production digestibility estimated through cumulative gas production at 24 h (equation 43f in Menke and Steingass, 1988).

⁶Estimated through molar proportion of VFA (Owens and Goetsch, 1988).

Dietary treatments affected total gas production measured over 24 and 48 h (Table 6). In the first 24 h, the SBM diet produced more gases compared with the CM diets, probably because of greater OM digestibility as previously discussed. However, at 48 h, when the substrate had more time for fermentation, only the HCM diet showed lower gas production ($P < 0.01$) compared with the SBM and LCM diets. These results also may be explained by OM digestibility, which was lower for the HCM diet than for the LCM diet ($P < 0.01$) after 24 h of digestion.

The SBM diet tended to increase CH₄ production (mM and g/kg of DM), which according to Owens and Goetsch (1988) may be associated with greater butyrate concentrations because for each 2 mol of butyrate produced there is a positive balance of 1 mol of CH₄. It was also observed that BCVFA concentration was greater on the SBM diet. Our data are in accordance with Hino and Russell (1985), who observed that ruminal H₂ and CH₄ increase during oxidative deamination and decarboxylation of branched-chain AA to form BCVFA in the rumen.

The SBM diet in experiment 1 (fed every 6 h) tended to result in a lower pH ($P = 0.10$) than the CM diets, whereas in experiment 2 the final pH (after 48 h of fermentation) was significantly lower for the SBM diet

($P < 0.01$). A lower pH may increase H₂ in the medium, resulting in more ATP and CH₄ production (Nelson et al., 2008). As a result, butyrate concentration tended to increase ($P = 0.06$) with the pH decrease on the SBM diet. When comparing CH₄ production in grams per kilogram of digested OM, no differences were observed among the diets ($P > 0.05$), which indicated that CH₄ production was more correlated with amount of substrate fermented than with the specific chemical composition of the diets.

Experiment 3

The main objective of experiment 3 was to evaluate ruminal gas production kinetics, in vitro OM digestibility, and CH₄ production of 3 protein supplements used in experiments 1 and 2. Gas production profiles during 48 h of fermentation of protein supplements are presented in Figure 2, and least squares means are presented in Table 7. Lag phase in this experiment was also considered as described earlier. It was observed that SBM and 38% RUP CM had a greater rate of gas production per hour than 50% RUP CM (0.11, 0.10, and 0.09 mL/h, respectively; $P < 0.01$), and SBM had greater gas pool size than both 38% RUP CM and 50% RUP CM (281, 239, and 238 mL/g of DM, respectively;

Figure 2. Ruminal gas production profiles of the 3 protein supplements (experiment 3). SBM = solvent soybean meal; CM = canola meal.

$P < 0.01$), which reflects the greater digestibility of the SBM compared with CM ingredients evaluated in these experiments.

When the protein supplements were evaluated separately, greater CH_4 production was also observed (mM and g/kg of DM) for SBM compared with both CM diets, which may be associated with the greater OM digestibility discussed previously. The SBM supplement had a greater concentration of total VFA and tended to have a lower propionate molar proportion than both CM supplements, which may be related to greater CH_4 production and total gas produced after 24 and 48 h of incubation for each gram of DM incubated. Another factor that could explain the greater values of CH_4 and total gas production for SBM is the greater concentration of BCVFA, as discussed previously. Greater CH_4 production is associated with greater energy losses and greater total gas produced after 24 and 48 h of incubation, which includes greenhouse gases. This may represent another advantage of CM compared with SBM and may explain why *in vivo* studies have shown that dietary CM may improve milk production when replacing SBM (Huhtanen et al., 2011; Broderick et al., 2015).

CONCLUSIONS

We hypothesized that CM with greater RUP content would change ruminal N metabolism compared with regular CM. The results from this study do not support the argument that changing CM RUP has an effect on ruminal N metabolism. It was observed that CM with

RUP varying from 38 up to 50% of CP did not affect ruminal N metabolism in a dual-flow continuous culture system. Furthermore, no major differences in ruminal N metabolism and digestibility were found between SBM and CM diets, which indicates that replacing SBM with CM has no major ruminal effects. This suggests that positive production responses previously observed when CM replaced SBM may have been attributable to postruminal effects and DMI. Minor ruminal effects observed in this study included decreasing isovalerate and BCVFA and increasing valerate molar proportions when CM replaced SBM. Despite lower CH_4 production (mM and g/kg of DM) for both CM diets compared with SBM, no differences in CH_4 g/kg of DM or CH_4 g/kg of digestible OM were found among the 3 diets.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the partial funding support from the Canola Council of Canada (Winnipeg, MB, Canada). They also thank the farm crew at the Main Station Field Laboratory (University of Nevada, Reno) for animal feeding and care.

REFERENCES

- AFRC (Agricultural and Food Research Council). 1993. Energy and Protein Requirements of Ruminants. CAB International, Wallington, UK.
- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, and T. Varvikko. 1999. Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. *Acta Agric. Scand. A* 49:179–189.
- Allison, M. J. 1969. Biosynthesis of amino acids by ruminal microorganisms. *J. Anim. Sci.* 29:797–807.
- Allison, M. J., and M. P. Bryant. 1963. Biosynthesis of branched-chain amino acids from branched-chain fatty acids by rumen bacteria. *Arch. Biochem. Biophys.* 101:269–277.
- AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
- AOAC International. 2005. Official Methods of Analysis. 18th ed. AOAC International, Arlington, VA.
- Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 88(E Suppl. 1):E9–E21.
- Bach, A., M. Ruiz Moreno, M. Thrune, and M. D. Stern. 2008. Evaluation of the fermentation dynamics of soluble crude protein from three protein sources in continuous culture fermenters. *J. Anim. Sci.* 86:1364–1371.
- Bach, A., and M. D. Stern. 1999. Effects of different levels of methionine and ruminally undegradable protein on the amino acid profile of effluent from continuous culture fermenters. *J. Anim. Sci.* 77:3377–3384.
- Benedetti, P. D. B., L. G. da Silva, E. M. de Paula, T. Shenkoru, M. I. Marcondes, H. F. Monteiro, B. Amorati, Y. Yeh, S. R. Poulson, and A. P. Faciola. 2015. Effects of partial replacement of corn with glycerin on ruminal fermentation in a dual-flow continuous culture system. *PLoS ONE* 10:e0143201.
- Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. *J. Dairy Sci.* 90:1816–1827.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007. Effects of different protein supplements on omasal nutrient flow and microbial

- protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1828–1841.
- Broderick, G. A., S. Colombini, S. Costa, M. A. Karsli, and A. P. Faciola. 2016. Chemical and ruminal in vitro evaluation of Canadian canola meals produced over 4 years. *J. Dairy Sci.* 99:7956–7970.
- Broderick, G. A., A. P. Faciola, and L. E. Armentano. 2015. Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. *J. Dairy Sci.* 98:5672–5687.
- Broderick, G. A., R. J. Wallace, and E. R. Orskov. 1991. Control of rate and extent of protein degradation. Pages 541–592 in *Physiological Aspects of Digestion and Metabolism in Ruminants*. T. Tsuda, Y. Sasaki, and R. Kawashima, ed. Academic Press, San Diego, CA.
- Calsamiglia, S., M. D. Stern, and J. L. Firkins. 1996. Comparison of nitrogen-15 and purines as microbial markers in continuous culture. *J. Anim. Sci.* 74:1375–1381.
- Canola Council of Canada. 2015. *Canola Meal Feeding Industry Guide*. 5th ed. Canola Council of Canada, Winnipeg, MB, Canada.
- Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8:130–132.
- Demeyer, D. 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. Pages 217–237 in *Rumen Microbial Metabolism and Ruminant Digestion*. J. P. Jouany ed. INRA Editions, Paris, France.
- Gidlund, H., M. Hetta, S. Krizsan, S. Lemosquet, and P. Huhtanen. 2015. Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage-based diets. *J. Dairy Sci.* 98:8093–8106.
- Hedqvist, H., and P. Udén. 2006. Measurement of soluble protein degradation in the rumen. *Anim. Feed Sci. Technol.* 126:1–21.
- Hino, T., and J. B. Russell. 1985. Effect of reducing-equivalent disposal and NADH/NAD on deamination of amino acids by intact rumen microorganisms and their cell extracts. *Appl. Environ. Microbiol.* 50:1368–1374.
- Hoover, W. H., B. A. Crooker, and C. J. Sniffen. 1976. Effects of differential solid-liquid removal rates on protozoa numbers in continuous cultures of rumen contents. *J. Anim. Sci.* 43:528–534.
- Huhtanen, P., M. Hetta, and C. Swensson. 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. *Can. J. Anim. Sci.* 91:529–543.
- Institut national de la recherche agronomique (INRA). 2007. *Nutrition of Cattle, Sheep and Goats: Animal Needs—Values of Feeds*. Éditions Quae, Versailles, France.
- Martineau, R., D. R. Ouellet, and H. Lapierre. 2013. Feeding canola meal to dairy cows: A meta-analysis on lactational responses. *J. Dairy Sci.* 96:1701–1714.
- Maxin, G., D. R. Ouellet, and H. Lapierre. 2013. Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability. *J. Dairy Sci.* 96:7806–7817.
- Menke, K. H., and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Develop.* 28:7–55.
- Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* 85:1217–1240.
- Nelson, D. L., A. L. Lehninger, and M. M. Cox. 2008. *Lehninger Principles of Biochemistry*. 5th ed. Macmillan, London, UK.
- National Research Council (NRC). 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academies Press, Washington, DC.
- Owens, F., and A. Goetsch. 1988. Ruminal fermentation. Pages 145–171 in *The Ruminant Animal. Digestive Physiology and Nutrition*. D. C. Church, ed. Prentice-Hall, Englewood Cliffs, NJ.
- Paula, E. M., M. A. C. Danes, N. E. Lobos, F. L. Drago, G. I. Zanton, G. A. Broderick, and A. P. Faciola. 2016a. Effects of replacing soybean meal with canola meal or treated canola meal on ruminal digestion, and omasal nutrient flow in lactating dairy cows. *J. Dairy Sci.* 99(E-Suppl. 1):668–669. (Abstr.)
- Paula, E. M., M. A. C. Danes, N. E. Lobos, G. I. Zanton, G. A. Broderick, and A. P. Faciola. 2016b. Effects of replacing soybean meal with canola meal or treated canola meal on nitrogen metabolism and total tract digestibility in lactating dairy cows. *J. Dairy Sci.* 99(E-Suppl. 1):757–758. (Abstr.)
- Pirt, S. J. 1975. *Principles of Microbe and Cell Cultivation*. Blackwell Scientific, Hoboken, NJ.
- Ramin, M., and P. Huhtanen. 2013. Development of equations for predicting methane emissions from ruminants. *J. Dairy Sci.* 96:2476–2493.
- Reynal, S. M., and G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 88:4045–4064.
- Reynal, S. M., I. R. Ipharraguerre, M. Lineiro, A. F. Brito, G. A. Broderick, and J. H. Clark. 2007. Omasal flow of soluble proteins, peptides, and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. *J. Dairy Sci.* 90:1887–1903.
- Sanchez, J. M., and D. W. Claypool. 1983. Canola meal as a protein supplement in dairy rations. *J. Dairy Sci.* 66:80–85.
- SAS Institute. 2002. *SAS User's Guide for Windows Environment 9.0*. SAS Institute, Cary, NC.
- Silva, L. G., J. Bunkers, E. M. Paula, T. Shenkoru, Y. Yeh, B. Amorati, D. Holcombe, and A. P. Faciola. 2016. Effects of flaxseed and chia seed on ruminal fermentation, nutrient digestibility, and long-chain fatty acid flow in a dual-flow continuous culture system. *J. Anim. Sci.* 94:1600–1609.
- Tagliapietra, F., M. Cattani, H. H. Hansen, I. K. Hindrichsen, L. Bailoni, and S. Schiavon. 2011. Metabolizable energy content of feeds based on 24 or 48h in situ NDF digestibility and on in vitro 24h gas production methods. *Anim. Feed Sci. Technol.* 170:182–191.
- Van Soest, P. J., and R. McQueen. 1973. The chemistry and estimation of fibre. *Proc. Nutr. Soc.* 32:123–130.
- Weller, R. A., and A. F. Pilgrim. 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. *Br. J. Nutr.* 32:341–351.
- Werner, R. A., B. A. Bruch, and W. A. Brand. 1999. Conflo III—An interface for high precision delta¹³C and delta¹⁵N analysis with an extended dynamic range. *Rapid Commun. Mass Spectrom.* 13:1237–1241.