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Effectiveness of canola meal as a source of rumen-undegraded protein for dairy cows

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ABSTRACT

Canola is an offspring of rapeseed (*Brassica napus* and *Brassica campestris/rapa*), canola seed is rich in oil, and after oil extraction, the remaining "*canola meal*" (CM), is a rich protein source used as feedstock to different animal species, mainly dairy cows in North America and in Europe. Despite the positive responses in milk production and nitrogen (N) utilization efficiency observed when soybean meal (SBM) is replaced with CM as the main protein supplement in dairy cow diets, it is unclear if the responses are due to a ruminal effect, a post ruminal effect, or a combination of both. The objectives of the research presented here were: 1) to evaluate whether the positive responses in milk production and N utilization efficiency are due to a better ruminal digestibility and N metabolism when CM is compared to SBM in dairy cow diets; 2) to evaluate whether these positive responses may be due to a greater contribution of the rumen undegraded protein (RUP) fraction and/or an increase of microbial protein synthesis to the metabolizable protein supply; and 3) to assess the potentially digestible neutral detergent-fiber (pdNDF) and the energy content of CM in a large sample set. To assess these objectives, first an in vitro study was performed (Chapter 2) evaluating the effects of feeding CM with different RUP content on ruminal fermentation, nutrient digestion, and microbial growth using a dual-flow continuous culture system. For this study, it was observed that CM with RUP varying from 38 up to 50% of CP did not affect ruminal N metabolism. Furthermore, no major differences in ruminal N metabolism and digestibility between SBM and CM diets were observed, which indicate that there are no major ruminal effects of replacing SBM with CM. Then a follow up in vivo study was performed (Chapter 3) to evaluate whether treating CM by extrusion to increase its RUP

content would improve RUP flow to the small intestine, N utilization and performance of dairy cows compared to regular CM and SBM. For this study, our results indicate that treating CM by extrusion was not effective in improving CM utilization by lactating in dairy cows. Nonetheless, when compared to the SBM diet, both CM diets decreased milk urea nitrogen (MUN) and N excretion in feces and urine. A third study was performed (Chapter 4) to assess whether the pdNDF of CM is underestimated based on current prediction models, and consequently its energy content is also underestimated. For this study, our results indicate that the pdNDF and the energy content are underestimated in current nutritional models. As an overall conclusion, our results indicate that the positive production responses previously observed when CM replaced SBM may have been due to post-ruminal effects and/or dry matter intake. Furthermore, treating CM by extrusion was not effective in improving CM utilization by lactating in dairy cows. However, CM may reduce the environmental impact compared to SBM, due to a lower urea N excretion as a proportion of total urinary N. More accurate information on CM NDF digestibility may improve energy content estimation, thus improving diet formulation accuracy.

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Chapter 1:

Overall Introduction

Canola is an offspring of rapeseed (*Brassica napus* and *Brassica campestris/rapa*) which was bread through standard plant breeding techniques to have low levels of erucic acid (< 2%) in the oil portion and low levels of glucosinolates (< 30 µmol/g) in the meal portion. The canola seed is rich in oil (approximately 42-43%), which is extracted for use as a vegetable oil. After oil extraction, the remaining "*canola meal*", is a rich protein source (approximately 40-43%) used as feedstock for monogastric and ruminant animals (Canola Meal Feed Guide, 2015). The reasons that glucosinolates and erucic acid were reduced in rapeseed is because they are toxic and may affect digestion and health of most animals (Kramer et al., 1990; Mawson et al., 1994), and consequently can limit CM inclusion levels in animal diets to very low amounts.

Before the genetic improvement achieved by Canadian plant breeders, rapeseed oil contained between 25-45 % erucic acid and 50-100 µmol of glucosinolate (Bell, 1993). *"The term "canola" (Canadian oil) was created in order to differentiate it from rapeseed. Some countries, especially in Europe, use the term "double-zero rapeseed" (low erucic acid, low glucosinolates) to identify "canola quality" seed, oil, and meal* (Canola Meal Feed Guide, 2015).

Canola seed is traditionally crushed and solvent extracted in order to separate the oil from the meal, by a process called pre-press solvent extraction. Meal quality is influenced by several variables during the process, mainly temperature. For instance, processing at elevated temperatures may reduce animal digestibility and amino acids (AA) availability, especially lysine (Newkirk et al., 2003).

Soybean meal (SBM) is the most commonly used protein supplement worldwide in dairy cow diets (Huhtanen et al., 2011). Soybean meal is characterized by well-balanced and available essential AA (EAA) contents (Awawdeh et al., 2007). In addition, SBM was ranked the second highest after microbial protein in EAA index, that take into account a utilization factor for each AA, and list the three most limiting EAA for each source (Santos et al., 1998). This makes SBM a high-quality protein supplement in dairy cow diets. In the northern latitudes where soybean (*Glycine max*) do not grow well, canola (rapeseed) is well adapted and presents high yield, and is a common protein supplement in this region as an alternative to soybean (Huhtanen et al., 2001).

In the 70s rapeseed meal (RSM) was becoming an increasingly important source of protein supplement in Canada. However, concerns were raised in regards to canola use as feed to animals due its glucosinolates and erucic acid content (Laarveld and Christensen, 1976). Glucosinolates may cause deleterious effects in animal health and production, such as, reduction in dry matter intake, induce iodine deficiency, and hypertrophy of liver, kidney, and thyroid when glucosinolates are consumed in large quantities (Tripathi and Mishra, 2007).

To address this concern and to evaluate the potential of canola/rapeseed meal as protein supplement in dairy cow diets several studies were conducted comparing different RSM varieties with themselves and with SBM. Iwarrson (1973) did not observe effects on blood parameters feeding high glucosinolates Swedish RSM at up to 8% in the diet of lactating dairy cows. On the other hand, Ingalls (1974) did observe a decrease in dry matter intake for cows fed a diet with RSM (high glucosinolates), compared to cows fed Bronowski RSM (low glucosinolates, low erucic acid) and SBM diets. In addition,

Laarveld and Christensen (1976) did not observe differences in yields of milk and composition, molar proportions of volatile fatty acids (VFA), and blood parameters correlated with hypothyroidism for diets with low glucosinolate RSM variety and SBM. Furthermore, Ingalls and McKirdy (1974) adding up to 19% low glucosinolate RSM variety in the total diet did not observe difference in dry matter intake, milk production and ruminal fermentation compared to diets with SBM.

Despite the results showing no difference in cows' performance when comparing the replacement of SBM with canola/rapeseed meal, producers have preferred SBM as a protein supplement in dairy cow diets, because canola/rapeseed meal has a greater fiber content and lower metabolizable energy than SBM. For this reason, the breeding program for canola/rapeseed was focused on developing varieties with low glucosinolate and low fiber content in the late seventies (Papas et., 1978).

Papas et al. (1978) evaluated the replacement of SBM with low fiber rapeseed and low indolyl-glucosinolate levels variety (1821 rape) or another rapeseed variety (with greater fiber) in diets of dairy cows. In addition, in a second experiment the authors evaluated the inclusion (4 or 8%) of rape gums, a by-product of the rape oil industry, as a source of energy in the rapeseed meal with the goal of increasing its energy content compared with SBM diets. The overall conclusion of their study was that replacing SBM with either 1821 rape (lower fiber) or rapeseed (greater fiber) resulted in similar milk production and composition, and feed intake. However, as the 1821 rape variety had lower protein content compared to the rapeseed (greater fiber), it was required to include a greater amount to balance the protein content of the diet, as consequence, this partially

diminished the advantage of having a lower fiber rapeseed meal. In addition, inclusion of gums had no effect on the performance of the cows.

The genetic improvement achieved by Canadian plant breeders reducing glucosinolates content in canola meal (CM), allowed the use of CM as protein supplement in different animal species, especially for ruminants. For example, among the different animal species, CM is mainly used in dairy cow diets in North America and in Europe (Arntfield and Hickling, 2011). However, according to Huhtanen et al. (2011), dairy farmers still have preference for SBM in the diet than CM. This is because SBM has a greater concentration of CP (53 vs. 42 % of dry matter), and greater metabolizable energy (3.41 vs. 2.75 Mcal/kg) compared to CM according to NRC (2001). In addition, feed evaluation systems, such as Agricultural and Food Research Council (AFRC 1993) and NRC (2001) estimate lower amount of ruminally undegraded protein (RUP) outflow and greater degradation rates of ruminally degraded protein (RDP) for CM compared to SBM, consequently the estimated metabolizable protein (MP) is also lower for CM.

Recently, studies evaluating the replacement of CM with SBM or other commonly protein supplements fed to dairy cows have shown an increase in cows' performance and an overall improvement in nitrogen (N) utilization for cows fed CM. Broderick et al. (2015) observed an increase in dry matter intake (DMI), yields of milk and true protein, and improvement in milk nitrogen efficiency replacing SBM with CM in isonitrogenous diets formulated with corn and/or alfalfa silage as source of forages. Two meta-analyses based on results of published peer-reviewed journals reported an increase of yields of milk and milk components, a reduction in MUN, and an increase in plasma concentration of branched-chain amino acids (BCAA) for cows fed CM compared to other protein

supplements (Martineau et al., 2013, 2014). Furthermore, Huhtanen et al. (2011) in another meta-analysis evaluated the replacement of SBM with CM in isonitrogenous diets formulated from grass silage-based diets, and observed an increase in DMI and yields of milk and milk components for CM diets compared to SBM.

Despite the positive responses observed in recent studies when CM is used as the main protein supplement in dairy cow diets, it is unclear if the responses are due to a ruminal effect, a post ruminal effect, or a combination of both. There have been speculations that these positive responses may be due to a greater contribution of the RUP fraction and/or an increase of microbial protein synthesis to the MP supply, consequently improving the amino acid (AA) balance available for absorption when CM is fed (Arntfield and Hickling, 2011; Maxin et al., 2013a).

However, according to previous studies CM may not be an effective source of AA due to its extensive ruminal protein degradation rates compared to SBM (Kendall et al., 1991; Whight et al., 2005). In addition, Piepenbrink and Schingoethe (1998) evaluated the ruminal degradation, and AA composition of RUP from CM, blood meal, corn gluten meal, and menhaden fish meal using in-situ methodology. The authors observed that CM had the greatest RDP and the lowest RUP content compared to the other four protein supplements. On the other hand, Maxin et al. (2013b) also using in-situ methodology, observed lower CP degradability and greater RUP content for CM compared to SBM. The discrepancy between these studies may be due to methodological assessments of the protein fractions and degradation.

For instances, in-situ methodology assumes that soluble proteins, peptides, and AA are completed degraded in the rumen, which may not be always true (Hedqvist and Uden,

2006; Reynal et al., 2007). Furthermore, in situ methodologies may impose physical restrictions to feed within the porous bags in the rumen and contain microbial contamination in undigested residues (Beckers et al., 1995). In addition, in-situ methodology estimates ruminal CP degradability of sole ingredient, which do not allow to evaluate the interactions of the protein supplement with other ingredients that make up the total diet, which may affect protein degradation. To our knowledge, there is only one study comparing the effects of CM vs. SBM in the total diet on ruminal fermentation parameters, and on the flow of nutrients out of the rumen in lactating dairy cows. In this study, authors did not observe differences between CM and SBM diets for ruminal nutrients outflow (Brito et al., 2007). Therefore, more studies are needed to evaluate the possible interactions of CM in the total diet with other ingredients and if the positive responses in overall performance of the cows fed CM are due to a ruminal effect. Furthermore, we also believe that there is room to increase the nutritive value of CM by applying chemical or physical treatments with the goal of increasing its RUP content, and potentially increasing AA availability for absorption in the small intestine.

Ruminally synthesized microbial CP, RUP, and endogenous protein are the main component of the MP, and the main source of AA for the maintenance, growth, and lactation of dairy cow (NRC, 2001). The main source of AA for the dairy cows comes from the synthesis of microbial protein in the rumen. However, for high producing dairy cows, the supply of microbial protein cannot meet the requirements of MP, consequently supplementation with high quality feed proteins that escape ruminal degradation is essential to meet the demands of AA for milk and milk protein synthesis (Hedqvist and Uden, 2006).

There are different methods that can be applied in feeds to decrease the ruminal degradability of proteins, such as chemical or physical treatment. For instances, heat treatment causes partial protein denaturation and Maillard reaction that decreases feed protein degradation in the rumen.

To our knowledge most of the studies evaluating the responses of treated CM on the performance and ruminal fermentation of dairy cows have been performed with diets based on grass and/or legume forage or protein mixtures (i.e., typical European diets).

Shingfield et al. (2003) compared the effect of heat-treated rapeseed expeller with SBM for dairy cows fed grass silage based diets. The authors observed greater milk production, and plasma concentration of histidine, EAA and BCAA for CM diets compared to SBM diets, indicating a better supply and balance of absorbed AA. Rinne et al. (1999) did not observe an effect in milk production between rapeseed meal and heat-moisture-treated rapeseed cake. Furthermore, Rinne et al. (2015) evaluated dairy cow response of increasing levels of rapeseed meal and SBM expeller in a red clover/grass silage based diet. The authors concluded that inclusion of expeller rapeseed meal in grass/red clover diets are more appropriated than SBM diets, due to greater milk production and milk protein synthesis. However, few studies have reported the responses of treated CM on the performance and ruminal fermentation of dairy cows with diets based on corn and/or alfalfa silage (typical North American diets). Wright et al. (2005) compared diets with heat-treated CM, heated + lignosulfonate treated CM, and untreated CM in a corn silage and barley diet to lactating dairy cows and reported a significant increase in DMI and milk production for diets with heat + lignosulfonate treated CM compared to untreated CM. However, the authors did not evaluate ruminal fermentation

parameters nor ruminal nutrient outflow. Furthermore, according to Martineau et al. (2013) meta-analysis, type of forage (e.g., grass or legumes forages vs. corn or barley silage) was one factor that influenced the responses of replacing other protein supplements with CM. Therefore, studies measuring omasal nutrient and microbial protein flow when untreated or treated CM is fed as the major protein supplement in corn and/or alfalfa silage-based diets to lactating dairy cows are needed to better evaluate the effects of CM on ruminal fermentation and milk production in North American diets.

Another aspect that we believe that may increase the nutritive value of CM, is its fiber content. As mentioned before the fiber content of CM is greater than SBM, because the canola seed has a relatively high amount of hulls, which stays with the meal (Newkirk, 2011), consequently the energy content of CM is lower than SBM. However, there have been speculations about the possibility of inaccuracies in the current values for canola meal indigestible NDF and NDF digestibility. These inaccuracies may be due to the high lignin content of canola meal, which estimates NDF digestibility fairly low based on current prediction models such as the National Research Council model (NRC, 2001) and the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004). Therefore, assessing the NDF potentially digestible and the energy content of CM may indicate ways to improve NDF digestibility of CM and consequently better estimate it energy content.

Therefore, the overall objectives of the present dissertation were: 1) to evaluate the effects of feeding CM with different RUP concentration and SBM on ruminal nutrient digestion, nitrogen metabolism, and ruminal gas production kinetics using two in-vitro systems; 2) to evaluate the effects of SBM, CM, and treated-CM on ruminal digestion

and omasal nutrient flow, nitrogen metabolism and production performance of lactating dairy cows; and 3) to assess CM potentially digestible NDF and its energy content in a large sample set from 12 Canadian crushing plants harvested over 4-years (2011, 2012, 2013, and 2014).

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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

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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 content on ruminal fermentation, nutrient digestion, and microbial growth using a dual-flow continuous culture system (Experiment1); and 2) evaluate ruminal gas production kinetics, in vitro OM digestibility, and methane (CH_4) 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 fermenters in a replicated 3×3 Latin square. The only ingredient that differed among diets was the protein supplement, with the treatments being: (1) solvent-extracted soybean meal (SBM); (2) low-RUP solvent-extracted-CM (LCM, 38% RUP as % of CP); and (3) high-RUP solvent-extracted-CM (HCM, 50% RUP). Diets were prepared as three 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 CH_4 production. In experiment 1, N flow (g/d), efficiency of N use, efficiency of bacterial N synthesis, total VFA (m*M*), and molar proportion of acetate, propionate, and isobutyrate were not affected by treatments. There were tendencies for a decrease ruminal NH_3-N and increase molar proportion of butyrate for the SBM diet compared to both CM diets. Molar proportion of valerate was greater in both CM diets, whereas the molar proportion of isovalerate and total branched chain volatile fatty acids were lower for CM diets compared to SBM. In experiments 2

and 3 SBM had a greater gas pool size than both CM diets. The SBM diet increased in vitro OM digestibility; however, it also tended to increase CH_4 production (m*M* and g/kg of DM) compared to both CM diets. Based on the results of this study, CM with RUP varying from 38-50% of total 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 due to 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 years, that CM varies in RUP content, ranging from 43-51% of its CP (Broderick et al., 2016), which may have an impact on ruminal N metabolism as well as on post-ruminal N utilization.

Recent studies comparing CM with SBM have shown an increase in milk yield, 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 (NH_3-N) and branched-chain VFA (BCVFA) concentrations, and decreased methane (CH_4) emissions per kg of ECM (Broderick et al., 2015; Gidlund et al., 2015). The reasons why CM improves milk and milk protein production, 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 or not there is also a ruminal effect of feeding CM that plays a role at making CM a better protein source for dairy cows.

We know of no published studies reporting the effects of CM containing different RUP concentration on rumen metabolism and gas production. Therefore, we selected, from a sub-set of our previous survey study (Broderick et al., 2016), CM with the lowest (38%, LCM) and the greatest (50%, HCM) RUP contents to assess whether or not this difference would impact 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 to regular CM; and 2) both CM diets would change ruminal fermentation, N metabolism, and total gas production compared to 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 CH_4 production.

MATERIALS AND METHODS

Animal care and handling was approved by the University of Nevada, Reno Institutional Animal Care and Use Committee (IACUC protocol # 00588).

Experiment 1

Diets and Experiment Design. Three diets were formulated to meet or exceed the dairy NRC recommendations (NRC, 2001), using as reference a Holstein cow producing 44 kg of milk, 120 DIM, and weighing 660 kg of BW. 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 % of CP (NRC, 2001); (2) CM with the lowest RUP content (LCM), 38% of RUP (Broderick et al., 2016); and (3) CM with the greatest RUP content (HCM), 50% of 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 Willey Mill (Model #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 fermenters contained 21% of CP. Each fermenter was fed 72 g/d of DM equally divided in 4 meals at 0600, 1200, 1800 and 2400. Diets were randomly assigned within square to six dual-flow continuous culture fermenters in a replicated 3 × 3 Latin square arrangement with three 10-d experimental periods, consisted 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 fermenters (Omni-Culture Plus; Virtis Co. Inc., Gardiner, NY) originally developed by Hoover et al. (1976) and recently modified by Benedeti et al. (2015) and Silva et al. (2016) were used. Ruminal fluid was collected approximately 2 h after feeding from two rumen-cannulated steers (average BW of 550 kg). The donor steers were fed (DM basis) a 60:40 forage: concentrate diet, containing 60% grass hay, 27.5% corn ground, 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 were poured into a pre-warmed insulated vessel. The rumen fluid was pooled and homogenized, infused with N_2 to maintain the anaerobic environment and kept at 39°C in a 5,000-mL Erlenmeyer flask in a pre-heated water bath. The rumen fluid was poured into each of the pre-warmed fermenters until it cleared the effluent spout.

Fermenter contents were continuously stirred by a central propeller apparatus driven by magnets at the rate 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) were used to monitor the pH of each fermenter.

On day 5, effluent digesta (liquid and solid) were homogenized and samples were collected to determine the background ^{15}N abundance. Then, 0.077 g of $(^{15}NH_4)_2SO_4$ with 10.2% atom excess of ^{15}N (Sigma-Adrich Co., St. Louis, MO) was infused into each fermenter to instantaneously label the NH_3-N pool. Saliva was reformulated to contain 0.077 g/L of the enriched $(^{15}NH_4)_2SO_4$ to replace an isonitrogenous amount of urea to obtain a steady-state ^{15}N enrichment of the NH_3 pool in the fermenters (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 effluents containers were immersed in a chilled water bath at 2˚C and 20 mL of 50% H_2SO_4 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 fermenter were taken and homogenized for 1 min (T25 basics, IKA Works, Inc., Wilmington, NC 28405) and 500 mL were removed via vacuum system and stored at -20˚C for later analysis of DM, OM, CP, NDF, and ADF. Additionally, two subsamples of 10 mL were filtered through 8 layers of cheese cloth, preserved with 0.2 mL of 0.2 *N* sulfuric acid, centrifuged at 1,000 × g for 15 min at 4°C, and the supernatant decanted and stored at -20°C for subsequent ruminal NH_3-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 fermenter contents were strained through two layers of cheesecloth, and centrifuged at 1,000 × g for 10 min. Then, the supernatant was centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments®) at 20,000 × g for 20 min. Supernatant was discarded and bacterial pellets were freeze-dried and stored for further analysis of ^{15}N, N, and OM (Bach et al., 2008).

***Chemical Analyses*.** Feed and effluent samples were analyzed for DM (method 934.01), EE (method 920.85), and ash (method 938.08) according to AOAC (1990). Crude protein content of feed samples was determined using a Leco combustion nitrogen analyzer (method 990.13; AOAC, 2005). Organic matter content was calculated as the difference between DM and ash contents. For NDF, samples were analyzed, being treated with thermo-stable α-amylase, and sodium sulfite according to Mertens (2002) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY). For ADF, samples were sequentially analyzed according to Van Soest and McQueen (1973) and adapted for the Ankom^{200} Fiber Analyzer. Neutral detergent-insoluble CP (NDICP) was isolated by gravimetric determination using thermo-stable α-amylase and sodium sulfite followed by CP analysis (method 990.13; AOAC, 2005). Nonfiber carbohydrates concentration of the feed ingredients was calculated using the equation: NFC = 100 - (%NDF + %CP + %ether extract + %ash) + NDICP, according to NRC (2001).

Concentration of VFA of the effluent samples was determined with a gas chromatograph (Varian Model 3800; Varian, Inc., Walnut Creek, CA) equipped with a glass column [180 cm × 4 mm i.d.] packed with GP 10% SP-1200/1% H_3PO_4 on 80/100

Chromosorb WAW [Supelco, Bellefonte, PA], with N_2 used as a carrier gas at a flow rate of 85 mL/min. The oven, injection port, and detector port temperatures were 125°C, 175°C, 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 3028 elemental analyzer interfaced to a Micromass Isoprime stable isotope ratio mass spectrometer (Werner et al., 1999).

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

Sample ^{15}N enrichment (atom percent excess) was calculated as sample ^{15}N atom % - background ^{15}N atom %.

Bacterial N flow (g/d) = (nonammonia N (NAN) flow × atom percent excess of ^{15}N of effluent) / (atom percent excess of ^{15}N of bacteria); Bacterial efficiency = Bacterial N flow (g) / OM truly digested (kg). True ruminal (DM, OM, CP, NDF, ADF) digestibilities were calculated as follows (using DM as an example):

True DM digestibility (%) = (g dietary DM - (g effluent DM - g saliva DM - g bacterial DM)) / (g dietary DM) × 100.

Nitrogen flows were calculated as follows:

NH_3-N flow (g/d) = (effluent NH_3-N (mg/dL)) × (g of total effluent flow /100);

NAN flow (g/d) = (g of effluent N) - (g of effluent NH_3-N);

Dietary N flow (g/d) = (g of effluent NAN) - (g of effluent bacterial N);

RUP-N flow (g/d) = (total N flow) - (effluent bacterial N flow);

RDP-N supply (g/d) = (total N intake) - (RUP-N flow).

Experiment 2

Experimental Design and Substrates. This experiment aimed to evaluate ruminal gas production kinetics, in vitro OM digestibility and CH_4, and ATP production from the three diets used in Experiment 1. A gas production apparatus (Ankom RF Gas Production System, Ankom Technology®, Macedon, NY, USA) equipped with pressure sensors that were wirelessly connected to a computer was used. The experimental design was: 3 incubation runs × 3 diets × 6 bottles per treatment, plus 18 blank bottles (6 per run), totaling 72 units. Dietary treatments were similar to 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 these 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 number 2; Arthur H. Thomas Co., Philadelphia, PA). 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 39ºC 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 were collected, immediately

filtered through 4 layers of cheesecloth and held in pre-warmed thermal containers (39ºC). The rumen fluid was mixed with the buffer solution (1:2 v/v) in a water bath at 39°C under anaerobic conditions by flushing with N_2 (Menke and Steingass, 1988). Bottles were inoculated with 75 mL of rumen fluid/buffer mixture solution while keeping the bottle headspace continuously flushed with N_2. Bottles without feed samples, but with 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, USA) under controlled temperature and agitation (39°C and 80 RPM). The data acquisition software (Gas Pressure Monitor, Ankom technology, NY, USA) 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 at the end of the incubation (48 h), the solution pH was measured with an Accumet portable AP61 pH meter (Fisher Scientific, Atlanta, GA).

At the end of the incubation, subsamples of 10 mL were filtered through two layers of cheesecloth from half of the bottles (9 observations per treatment), and 0.2 mL of a 50% H_2SO_4 solution was added for later VFA determination and CH_4 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, CH_4, and ATP production from the three protein supplements as a sole ingredient. This experiment used the same gas production

apparatus system, experimental design, and the three protein supplements used in Experiments 1 and 2. The chemical composition of the ingredients is shown in Table 2. The three protein supplements were also ground to pass through a 1-mm screen in a Wiley Mill (model number 2; Arthur H. Thomas Co., Philadelphia, PA).

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 (Experiment 2 and 3). The three 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. VFA concentrations were determined using gas chromatography, as described in Experiment 1. Post fermentation freeze-dried samples were analyzed for DM, and subsequently for NDF.

Calculations (Experiment 2 and 3). The ATP and CH_4 production were estimated according to Owens and Goetsch (1988) through VFA stoichiometry using the following equations:

ATP = (1/4 Acetate) + (2 3/4 Propionate) + (3 1/2 Butyrate);

CH_4 = (1/2 Acetate) + (1/2 Butyrate) – (1/4 Propionate).

The in vitro true OM digestibility was calculated as:
iv-tOMd (%) = (iOM – rNDF)/(iOM), which iOM was the incubated OM and rNDF 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 (GP, mL) according to Tagliapietra et al. (2011) that used the ideal gas law, in which GP (mL) = (Pc/Po) × Vo, being Pc the cumulated pressure change (kPa) in the bottle

headspace, Vo the bottle headspace volume (545 mL), and Po 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 μ is 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 to 3), $F(S)_{ki}$ is the random effect of fermenter (F) within square (k = 1 to 6), D_l is the fixed effect of diet (l = 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: SBM vs. (LCM + HCM), and LCM vs. HCM. Least square means and SEM 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:

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

where μ is 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 to 3), $F(S)_{ki}$ is the random effect of fermenter (F) within square (k = 1 to 6), D_l is the fixed effect of diet (l = SBM, LCM or HCM), T_m is the fixed effect of time (m = 1 to 5), DT_{lm} is the interaction between diet and time, and E_{ijklm} is the residual error. The effect of fermenter 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, 2002), with a statistical model including fixed effect of treatment and random effect of run. Orthogonal contrasts as describe earlier in experiment 1. An exponential model with lag phase was fitted to the cumulative gas production using the NLIN procedure of SAS 9.4 (SAS, 2002) to analyze ruminal gas production kinetics over 48 h in Experiments 2 and 3. Least square means and SEM are reported for all data with a significance declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Ruminal pH and Nitrogen Metabolism. There were no differences in rumen pH across diets. The average for all dietary treatments was 6.4 with a minor variation between feed intervals (minimum 6.4 and 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, and RDP-N supply, efficiency of N utilization (ENU), and efficiency of bacterial N synthesis (Table 3). Although there was a trend ($P = 0.09$) for lower NH_3-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 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. In two meta-analysis studies

(Huhtanen et al., 2011; Martineau et al., 2013) reported an increase in DMI for diets supplemented with CM compared to 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, since 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 compared to SBM diets.

Although the high dietary CP might have played a role in ruminal N fermentation, especially with regards to RDP and NH_3-N levels, previous studies with 16.5 and 15.7% CP, respectively, reported similar results with regards to ruminal N metabolism when comparing SBM and CM (Brito and Broderick 2007 and Paula et al., 2016a). This suggests that dietary CP levels play a minor role when comparing these two 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 two CM tested (38 and 50% RUP, % 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 was 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 to CM due to 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, and this could be due to methodological assessments of RDP/RUP. For example, early studies reporting CM RDP/RUP fraction used in situ methodology, which assumes soluble proteins, peptides, and AA are completed degraded in the rumen, which may not be always the case (Reynal et al., 2007). Furthermore, Hedqvist and Uden (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) have 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/RDP values in feed tables may be due to differences in seed processing/crushing methods, for example, the NRC (2001) only lists mechanical extracted CM (expeller CM); however, solvent-extracted 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 to SBM. Broderick et al. (2015) observed a significant decrease in ruminal NH_3-N concentration for CM diet compared to 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 for each 1 kg of DMI, 79.8 g of RUP were consumed; whereas, for the HCM diet for each 1 kg of DMI, 105 g of RUP were consumed, so that 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 TMRs were fed, meaning that possible interactions among ingredients may affect protein degradation.

Volatile Fatty Acids. Total VFA concentration, molar proportions of acetate, propionate, and isobutyrate, and acetate: propionate ratio were not affected by dietary treatments (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 due to greater NFC content in the SBM compare to CM diets (41.3, 36.0, and 36.3%, respectively), which could contribute to more microbial fermentation compared to the CM diets. Testing SBM and CM as protein supplements, Brito and Broderick (2007) had diets differing in NFC content at approximately 4% units (50.2 and 46.4%, 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% units (47.8 and 44.5%, SBM and CM, respectively) and also 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.

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 (BCAA; valine, isoleucine, and leucine) oxidative deamination and decarboxylation (Allison and Bryant, 1963). Therefore, these BCVFA are associated with degradation of 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.

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, and 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 SBM and CM treatment, which agrees with our findings. In a review and meta-analysis evaluating CM as protein supplement for dairy cows Huhtanen et al. (2011) did observe reduced total tract true CP digestibility for heat-treated CM diets when compared to untreated CM. 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 only measured ruminal CP digestibility as opposed to total tract CP digestibility. Similarly 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 also did not observe 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 heat-treated CM diet compared to regular CM (Paula et al. 2016b). Therefore, it is likely that the lack of effects on ruminal CP digestibility between CM diets may be due 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 square 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 considered as apparent (Pirt, 1975). Although diets did not differ in rate of gas production (0.06 mL/h, average for all treatments; $P = 0.56$), SBM diet had a greater gas pool size (potentially fermentable fraction) than both CM diets (342 mL/g DM versus 306 and 309 mL/g DM, respectively for SBM versus LCM, and HCM diets; $P < 0.01$). However, LCM and HCM diets did not differ between each other ($P > 0.05$).

The greater gas pool size for SBM diet may have been due to a greater OM digestibility after 48 h compared to 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 SBM diet may have been due to a greater BCVFA concentration in SBM diet (data not shown), which may have contributed to the growth of cellulolytic and some non-cellulolytic bacteria (Allison, 1969).

Dietary treatments changed total VFA concentration and acetate and butyrate molar proportions (Table 6). Total VFA concentration increased in SBM diets compared to 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 to CM diets. These changes may be due to the conversion of acetate into butyrate, which may occurs when high soluble carbohydrates diets are fed (Demeyer, 1991). Similar results were observed in experiment 3 (Table 7).

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

The SBM diet tended to increase CH_4 production (m*M* and g/kg of DM), which according to Owens and Goetsch (1988) may be associated with greater butyrate concentrations as for each two moles of butyrate produced there is a positive balance of one mole of CH_4. 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 H_2 and CH_4 increase during oxidative deamination and decarboxylation of BCAA 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 CM diets, whereas in Experiment 2 the final pH (after 48 h of fermentation) was significantly lower for SBM diet ($P < 0.01$). A lower pH may increase H_2 in the medium resulting in more ATP and CH_4 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 CH_4 production in g/kg of digested OM, no differences were observed among the diets ($P > 0.05$), which indicated that CH_4 production was more correlated to amount of substrate fermented than to 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 CH_4 production of three protein supplements used in Experiment 1 and 2. Gas production profiles during 48 h fermentation of protein supplements are presented in Figure 2 and least square 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 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 ingredient had greater gas pool size than both 38%-RUP CM and 50%-RUP CM (281, 239, and 238 mL/g of DM, respectively; $P < 0.01$), which reflects the greater digestibility of the SBM compared to CM ingredients evaluated in these experiments.

When the protein supplements were evaluated separately, greater CH_4 production was also observed (m*M* and g/kg of DM) for SBM compared to 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 with greater CH_4 production, GP_{24}, and GP_{48}. Another reason 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 GP_{24} and GP_{48}, which include greenhouse gases, may represent another advantage of CM compared to SBM, this 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 to regular CM and SBM. The results from this study do not support the argument that changing CM RUP has an effect on ruminal 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, there were no major differences in ruminal N metabolism and digestibility between SBM and CM diets, which indicate that there are no major ruminal effects of replacing SBM with CM, suggesting that positive production responses previously observed when CM replaced SBM may have been due to post-ruminal effects and DMI. Minor ruminal effects observed in this study included decreasing isovalerate, BCVFA, and increasing valerate molar proportions

when CM replaced SBM. Despite lower CH_4 production (m*M* and g/kg of DM) for both CM compared to SBM, there were no differences in CH_4/DM or CH_4/fermented OM among the 3 diets.

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

	Diets		
Item	SBM	LCM	HCM
Orchard hay	25.0	25.0	25.0
Wheat straw	15.0	15.0	15.0
Corn ground	32.5	24.3	23.0
SBM[1]	25.0	-	-
LCM[2]	-	33.2	
HCM[3]	-	-	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[4]	14.5	13.2	12.9
RUP[4]	7.0	7.5	7.7
RDP[5]	13.3	13.1	12.6
RUP[6]	8.2	7.7	8.1
NDF	31.1	37.8	37.5
ADF	18.2	24.5	25.2
NFC[7]	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[8], Mcal/kg of DM	1.58	1.50	1.50

[1]SBM = solvent soybean meal.
[2]LCM = low-RUP canola meal diet.
[3]HCM = high-RUP canola meal diet.
[4]Estimated using the NRC (2001) model.
[5]RDP was calculated from in vitro dual-flow continuous culture measurements as RDP, % of DM = (total CP intake, g/d - effluent RUP flow, g/d) × 100/DM intake, g/d. Adapted from Reynal and Broderick (2005).
[6]RUP was calculated 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/DM intake, g/d. Adapted from Reynal and Broderick (2005).
[7]NFC = 100 – (%NDF + %CP + %fat + %ash) + %NIDN × 6.25 according to the NRC (2001) model.
[8]NE_L = Net energy for lactation, estimated using the NRC (2001) model.

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

	Protein Supplements[1]		
Chemical Composition	SBM	38%-RUP CM	50%-RUP CM
OM	92.7	92.5	91.8
CP	54.9	43.6	42.1
RDP, % of CP	31.52[2]	26.93[3]	21.23[3]
RUP, % of CP	23.42[2]	16.73[3]	20.93[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

[1]SBM = standard soybean meal; 38%-RUP CM = canola meal with 38% RUP; 50%-RUP CM = canola meal with 50% RUP.
[2]Estimated using the NRC (2001) model for a cow with DMI = 4 % of BW.
[3]Estimated according to Broderick et al. (2016).
[4]Non-fiber carbohydrates = 100 - (% NDF + %CP + %fat + %ash) + NDIN × 6.25, according to NRC (2001).
[5]Estimated using the NRC (2001) model.

Table 3. Effects of different protein sources on pH and nitrogen metabolism in dual-flow continuous culture (Exp. 1)

	Treatment[1]				*P*-value[2]	
Item	SBM	LCM	HCM	SEM	SBM vs. LCM+HCM	LCM vs. HCM
pH	6.40	6.50	6.43	0.07	0.10	0.11
NH_3-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
NH_3-N	0.51	0.56	0.53	0.02	0.09	0.19
NAN[3]	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[4]	0.94	0.88	0.93	0.05	0.57	0.54
RDP-N[5] supply	2.07	2.04	1.98	0.07	0.44	0.47
ENU[6], %	76.0	74.8	76.3	2.27	0.86	0.66
Bacterial efficiency[7]	46.5	45.7	46.7	2.45	0.90	0.77

[1]SBM = dietary treatment with solvent soybean meal as protein supplement; LCM = dietary treatment with 38% RUP canola meal as protein supplement; HCM = dietary treatment with 50% RUP canola meal as protein supplement.
[2]Orthogonal contrasts for effects of different protein supplement (SBM vs. LCM + HCM), and (LCM vs. HCM).
[3]NAN = nonammonia nitrogen.
[4]RUP-N= rumen undegradable protein nitrogen.
[5]RDP-N = rumen degradable protein nitrogen.
[6]ENU= Efficiency of N use = g of bacterial N/g of available N (Bach and Stern, 1999).
[7]Bacterial efficiency = g of bacterial N/kg of OM truly digested.

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

	Treatment[1]				*P*-value[2]	
Item	SBM	LCM	HCM	SEM	SBM vs. LCM+HCM	LCM vs. HCM
Total VFA, m*M*	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], m*M*	2.08	1.77	1.76	0.11	0.04	0.67

[1]SBM = dietary treatment with solvent soybean meal as protein supplement; LCM = dietary treatment with 38% RUP canola meal as protein supplement; HCM = dietary treatment with 50% RUP canola meal as protein supplement.
[2]Orthogonal contrasts for effects of different protein supplement (SBM vs. LCM + HCM), and (LCM vs. HCM).
[3]BCVFA = branched-chain VFA.

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

	Treatment[1]				*P*-value[2]	
	SBM	LCM	HCM	SEM	SBM vs. LCM+HCM	LCM vs. HCM
Ruminal true digestibility, %						
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

[1]SBM = dietary treatment with solvent soybean meal as protein supplement; LCM = dietary treatment with 38% RUP canola meal as protein supplement; HCM = dietary treatment with 50% RUP canola meal as protein supplement.

[2]Orthogonal contrasts for effects of different protein supplement (SBM vs. LCM + HCM), and (LCM vs. HCM).

Table 6. Effects of diets with different RUP content on in vitro gas production parameters and digestibility (Exp. 2)

	Treatment[1]				*P*-value[2]	
Item	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 DM	342	306	309	12.00	<0.01	0.61
Total GP_{24}[3], mL/g DM	260	248	225	9.30	<0.01	<0.01
Total GP_{48}[3], mL/g 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, m*M*	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], m*M*	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 dOM	40.8	41.5	40.1	5.26	0.99	0.63
CH_4, g/kg DM	34.1	30.5	30.3	2.33	0.07	0.93

[1]SBM = dietary treatment with solvent soybean meal as protein supplement; LCM = dietary treatment with 38% RUP canola meal as protein supplement; HCM = dietary treatment with 50% RUP canola meal as protein supplement.
[2]Orthogonal contrasts for effects of different protein supplement (SBM vs. LCM + HCM), and (LCM vs. HCM).
[3]Total gas produced after 24 and 48 h of incubation for each gram of dry matter incubated.
[4]In vitro true OM digestibility estimated after 48h of digestion.
[5]OM_{GP} digestibility: estimated through cumulative gas production at 24h (equation 43f, Menke and Steingass, 1988).
[6]ATP and CH_4 were estimated through volatile fatty acids production (Owens and Goetsch, 1988).

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

	Treatment[1]				*P*-value[2]	
Item	SBM	38%-RUP CM	50%-RUP CM	SEM	SBM vs. 38%-RUP CM +50%-RUP CM	38%-RUP CM vs. 50%-RUP CM
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 DM	281	239	238	8.6	<0.01	0.96
Total GP_{24}[3], mL/g DM	257	212	198	9.5	<0.01	0.13
Total GP_{48}[3], mL/g 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, m*M*	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], m*M*	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 dOM	41.6	40.9	38.2	1.88	0.30	0.18
CH_4, g/kg DM	37.5	30.3	28.3	1.38	<0.01	0.18

[1]SBM = Solvent soybean meal, 38%-RUP CM = 38%-RUP canola meal, 50%-RUP CM = 50%-RUP canola meal.
[2]Orthogonal contrasts for effects of different protein supplement (SBM vs. 38%-RUP CM + 50%-RUP CM), and (38%-RUP CM vs. 50%-RUP CM).
[3]Total gas produced after 24 and 48 h of incubation for each gram of dry matter incubated.
[4]In vitro true OM digestibility estimated after 48h of digestion.
[5]OM_{GP} digestibility: estimated through cumulative gas production at 24h (equation 43f, Menke and Steingass, 1988).
[6]ATP and CH_4 were estimated through molar proportion of VFA (Owens and Goetsch, 1988).

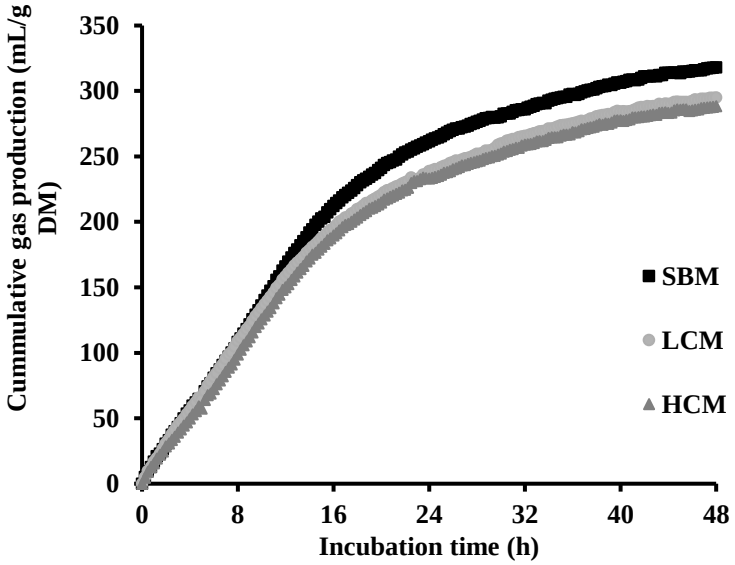

Figure 1. Ruminal gas production profiles of the diets used in experiments 1 and 2 determined with the Ankom gas production system. SBM = dietary treatment with solvent soybean meal as protein supplement; LCM = dietary treatment with 38% RUP canola meal as protein supplement; HCM = dietary treatment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

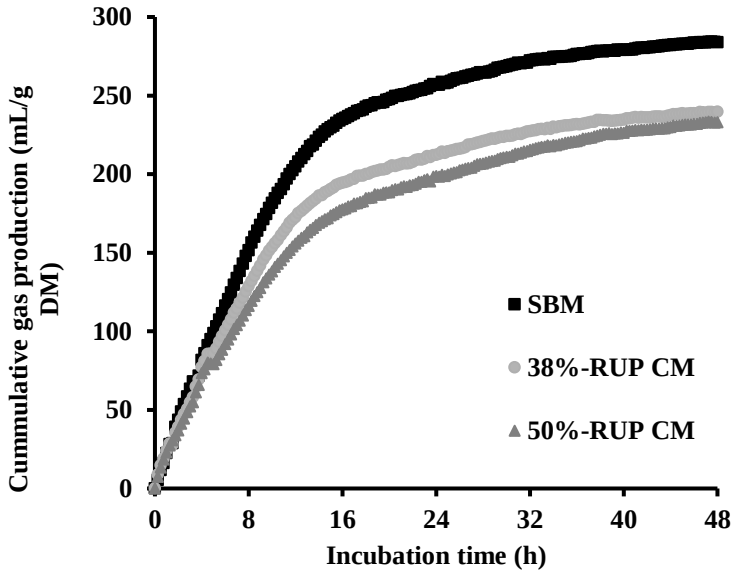

Figure 2. Ruminal gas production profiles of the three protein supplements (Exp. 3). SBM = Solvent soybean meal, 38%-RUP CM = 38%-RUP canola meal, 50%-RUP CM = 50%-RUP canola meal.

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Effects of replacing soybean meal with canola meal or treated canola meal on ruminal digestion, omasal nutrient flow, and performance in lactating dairy cows

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Abstract: Extrusion treated canola meal (TCM) was produced in an attempt to increase the rumen-undegraded protein (RUP) fraction of canola meal (CM). The objective of this study was to evaluate the effects of replacing soybean meal (SBM) with CM or TCM on ruminal digestion, omasal nutrient flow, and performance in lactating dairy cows. To assess performance, 30 multiparous Holstein cows averaging (means ± SD) 119 ± 23 days in milk (DIM) and 44 ± 7 kg milk/d, and 15 primiparous cows averaging 121 ± 19 DIM, and 34 ± 6 kg milk/d were blocked in a randomized complete block design with a 2-week covariate period and 12-week experimental period (Experiment 1). Dietary ingredients differed only in protein supplements, which were: SBM, CM, or TCM. All diets were formulated to contain (DM basis) 30% alfalfa silage, 30% corn silage, 4% soy hulls, 2.4% mineral-vitamin premix, and 16% CP. The SBM diet contained 25% High-moisture shelled corn (HMSC) and 8.6% SBM; the canola diets contained 22% HMSC and 11.2% CM or 11.4% TCM. To assess ruminal digestion, and omasal nutrient flow, six rumen-cannulated cows were blocked into 2 squares of 3 cows and randomly assigned within blocks to the same 3 dietary treatments as in Experiment 1, in a replicated 3×3 Latin square design (Experiment 2). Data were analyzed using the MIXED procedure of SAS. Orthogonal contrasts were used to compare effects of different protein supplements (SBM vs. CM + TCM) and (CM vs. TCM). In experiment 1, compared to SBM, apparent total tract digestibilities of dry matter (DM) and nutrients were greater in cows fed both CM diets and there was a tendency for nutrient digestibilities to be higher in cows fed CM compared TCM. Diets did not affect milk yield and milk components; however, both canola diets decreased urinary urea-N (% of total urinary N), fecal-N (% of total N intake), and milk urea N (MUN) concentration. In

experiment 2, compared to SBM, both canola diets increased N intake and tended to increase rumen-degraded protein (RDP) supply (kg/d) and N truly digested in the rumen (kg/d). Diets did not affect ruminal digestibility, efficiency of microbial protein synthesis, and RUP flow among diets. Results from this experiment indicate that replacing SBM with CM or TCM in diets of lactating cows improved digestibility and may reduce environmental impact. Moreover, under the conditions of the present study, treating CM by extrusion did not improve CM utilization.

Key words: canola meal, extrusion, nitrogen metabolism, rumen-undegraded protein

INTRODUCTION

Canola meal (**CM**) is widely used in North America as a protein supplement for lactating dairy cows. Previous studies comparing the effects of soybean meal (**SBM**) or other protein supplements vs. CM have shown that CM diets increase nitrogen (**N**) utilization and performance when fed to lactating dairy cows (Huhtanen et al., 2011; Broderick et al., 2015). Improvement in N utilization and performance using CM in dairy cows diets may be due to an increase in microbial protein synthesis and/or a greater MP supply from the RUP fraction (Maxin et al., 2013a). However, it is still unclear if responses observed when CM is fed are due to a ruminal effect, a post-ruminal effect, or a combination of both.

Chemical and physical treatment are strategies used to increase the RUP fraction of protein supplements with the goal of increasing AA availability for absorption in the small intestine and consequently optimizing the performance of dairy cows (Santos et al., 1998; Huhtanen et al., 2011). This strategy has been widely used with SBM, and as a consequence, many SBM-based commercial products have been developed. However, studies evaluating the response of treated CM on the performance of dairy cows have been inconsistent. For instance, Rinne et al. (1999) did not find an effect in yields of milk between rapeseed meal and heat-moisture-treated rapeseed cake. Conversely, Wright et al. (2005) found an increase in milk yield for cows fed heat-treated CM plus lignosulfonate compared to untreated CM.

In a meta-analysis, Huhtanen et al. (2011) did not find differences in DMI, digestibility, and milk yield comparing regular CM vs. heat-treated CM. Paula et al. (2017) did not find differences in RUP and microbial protein synthesis flow comparing

CM differing in RUP content in an in vitro study using a dual-flow continuous culture system. Furthermore, Ahvenjärvi et al. (1999) did not observe differences in omasal NAN and microbial NAN flow in cows fed either rapeseed meal or heat-moisture-treated rapeseed cake. Similarly, Khorasani et al. (1993) did not find differences in ruminal CP degradability in cows fed CM or CM treated with acetic acid. However, to our knowledge, there are no studies measuring omasal nutrient and microbial protein flow when treated CM is fed as the major protein supplement in corn and/or alfalfa silage-based diets to lactating dairy cows.

In the present study, CM was treated by extrusion in an attempt to increase its RUP content. We hypothesized that: 1) feeding treated CM (**TCM**) would improve RUP flow to the small intestine, N utilization and performance of dairy cows compared to regular solvent-extracted CM; and 2) both CM diets would improve N utilization and performance of dairy cows compared to regular solvent-extracted SBM. Therefore, the objectives of the present study were to evaluate the effects of SBM, CM, and TCM on: 1) ruminal digestion and omasal nutrient flow; 2) to measure total tract digestibility, N metabolism and production performance of lactating dairy cows.

MATERIAL AND METHODS

Care and handling of all experimental animals, including ruminal cannulation, were conducted under protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Experiment 1. Experiment 1 was designed to avoid potential carry-over effects from reversal experiments; moreover, it allowed cows to stay on the same diet longer,

which could have the potential to better reflect the effects of the protein supplement on milk production. Thirty multiparous Holstein cows averaging, at the beginning of the study, (means ± SD) 2.5 ± 0.6 parity, 660 ± 55 kg BW, 119 ± 23 DIM, and 44 ± 7 kg milk/d, and 15 primiparous cows averaging (means ± SD) 592 ± 34 kg BW, 121 ± 19 DIM, and 34 ± 6 kg milk/d were blocked into groups of three by parity and DIM to obtain 10 multiparous blocks and five primiparous blocks in a randomized complete block design study. Cows were fed a control diet for a 2-week covariate period and then switched to the experimental diets for a 12-week study. The control diet contained (DM basis) 30% alfalfa silage, 30% corn silage, 22.2% High-moisture shelled corn (**HMSC**), 4.3% SBM, 5.8% regular CM, 5.3% soy hulls, and 2.4% mineral-vitamin premix. The CM was treated by extrusion, with added molasses (2 to 3%) to promote the browning reaction. The meal was preconditioned with steam containing reducing sugars and a proprietary blend of carbohydrases to a moisture level of 15%, then treated using a high temperature-short time annular gap expander (Kahl GmbH, Reinbek, Germany). The expander had a cone pressure of 13 bars. The same batch of meal was used for CM and TCM during the entire experimental period. Chemical composition of the fermented feeds and protein supplements fed is shown in Table 1. All diets contained (DM basis) 30% alfalfa silage, 30% corn silage, 4% soy hulls, 2.4% mineral-vitamin premix, plus one of the following protein supplements: SBM (8.4%), CM (11.2%), or TCM (11.4%). High-moisture shelled corn was decreased from 25% in the SBM diet to 22% in both CM diets. Dietary CP contents were approximately 16% for all diets (Table 2).

All cows were injected biweekly with rbST (500 mg of Posilac, Elanco Animal Health, Greenfield, IN). Cows were housed in tie stalls and had free access to water during the experiment.

Diets were offered once daily at 1000 h. Orts were collected and weights recorded at 0900 h and feeding rate was adjusted daily to yield orts between 5 to 10% of intake. Weekly composite samples (500 g) were taken from daily samples of corn silage, alfalfa silage, HMSC, TMR, and orts and stored at -20°C until analysis. Weekly samples of SBM, CM, TCM, and soy hulls were also taken and stored at room temperature. The DM content was determined in weekly composites of corn silage, alfalfa silage, and HMSC by drying at 60°C for 48 h and in weekly samples of soybean meal, canola meal, treated CM, and soy hulls at 105°C, according to AOAC (1990). Weekly samples of feed ingredients were also analyzed for total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI), according to AOAC (2005) (method 990.13). Ingredient DM and N were used to adjust dietary composition weekly to maintain constant DM proportions from each feed ingredient and CP contents in each diet. The DMI was computed daily based on the 60°C DM determinations for TMR and orts. After drying, ingredients and TMR were ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for DM (method 934.01), ash and OM (method 938.08) according to AOAC (1990), and total N as previously described. Samples were sequentially analyzed for NDF and ADF, after being treated with thermo-stable α-amylase and Na_2SO_3 according to Van Soest et al. (1991) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY).

Indigestible ADF was analyzed in the ADF residue remaining after 12-d in situ incubation according to Huhtanen et al. (1994).

Cows were milked three times daily at 0300, 1100, and 1700 h and milk yield was recorded at each milking time during the entire experiment. Milk samples from all 3 daily milkings were collected on the last 4 d of weeks 4, 6, 8, 10, and 12 and were analyzed for fat, true protein, lactose, SNF, and MUN by infrared analysis (AgSource, Verona, WI) with a spectrum analyzer (FT6000; Foss North America Inc., Eden Prairie, MN) using AOAC (1990) method 972.16. Concentrations and yields of fat, true protein, lactose, SNF, and MUN were calculated as weighted means based on morning, afternoon, and evening milk yields on each test day. Yields of 3.5% FCM were calculated according to Sklan et al. (1992) and yields of ECM were calculated as described by Krause and Combs (2003).

Efficiencies of feed conversion were calculated for each cow over week 4, 6, 8, 10, and 12 by dividing the average yield of actual milk and ECM by the respective DMI. Efficiency of feed N utilization was calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean milk N intake, assuming no net deposit or mobilization of N from body tissues.

On last day of week 4, 8, and 12, 2 spot urine and 2 spot fecal samples were collected from each cow 6 h before and 6 h after feeding. Fecal samples were dried in a forced-draft oven (60°C; 72 h) and ground to pass a 1-mm screen (Wiley mill). Equal DM from each fecal subsample was combined to obtain one composite sample for each cow in week 4, 8, and 12. Fecal samples were analyzed for total DM, ash, OM, N, NDF, ADF, and indigestible ADF as described for feed analysis. Indigestible ADF was used as

internal marker to estimate apparent total tract digestibility and fecal output, using the respective DMI, according to Cochran et al. (1986). Urine samples were acidified immediately after collection by diluting 1 volume of urine with 4 volumes of 0.072 *N* H_2SO_4 and stored at -20°C until analysis. Before the analysis, urine samples were thawed at room temperature and filtered through Whatman No. 1 filter paper. Filtrates were analyzed for creatinine using a picric acid method (Oser, 1965) adapted to flow-injection analysis (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI), and for total N using a N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc.). In addition, filtrates were analyzed for allantoin using the method of Vogels and Van der Drift (1970) adapted to a 96-well plate reader, and for uric acid using a commercial kit (No. 683-100P, Sigma Chemical Co., St Louis, MO), and for urea with the colorimetric method (Broderick and Clayton, 1997). Daily urine volume was calculated based on individual BW and using creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urinary urea N, total N, and total purine derivatives (PD), and allantoin plus uric acid, were calculated based on their individual daily excretion multiplied by daily urine volume.

Experiment 2

Experiment 2 was run in parallel with Experiment 1 with six ruminally cannulated cows to assess ruminal digestion and omasal nutrient flow. Four multiparous Holstein rumen-cannulated cows averaging, at the beginning of the study, (means ± SD) 694 ± 56 kg BW, 220 ± 71 DIM, and 34.7 ± 9 kg milk/d, and two primiparous Holstein rumen-cannulated cows averaging 680 ± 6 kg BW, 242 ± 2 DIM, and 32.6 ± 2 kg milk/d were used. Cows were randomly assigned to treatment sequences in a replicate 3 × 3 Latin

square design with 21 days of diet adaptation and 7 days of sampling. Dietary treatments were as described for experiment 1.

Omasal sampling was performed each period using the technique developed by Huhtanen et al. (1997) and Ahvenjärvi et al. (2000), and adapted by Reynal and Broderick (2005) to quantify digesta flow out of the rumen. External markers were used to estimate nutrient flow at the omasal canal: indigestible NDF (Huhtanen et al., 1994), $YbCl_3$ (Siddons et al., 1985), and Co-EDTA (Udén et al., 1980), were used as markers for large particles, small particles, and fluid phases of digesta, respectively. The external microbial marker ^{15}N was used to quantify microbial NAN flow from the rumen. Before marker infusion began, approximately 100 g of ruminal contents were taken from each cow to determine the background ^{15}N abundance. Cobalt-EDTA, $YbCl_3$, and $^{15}NH_4SO_4$ containing 10% atom excess ^{15}N (Isotec, Miamisburg, OH) were dissolved in distilled water and continuously infused into the rumen at rate of 2.6 g of Co-EDTA, 3.2 g of $YbCl_3$, and 231 mg of ^{15}N per d in 2.89 L/d of solution. Markers were continuously infused from d 22 to 28 using a peristaltic pump (AutoAnalyzer II, Technicon Corp., St. Louis, MO).

Beginning on d 26, omasal samples were collected at 12-time points in 2-h intervals over a 3-d period to represent a 24-h feeding cycle. Sampling protocols, including confirming that the sample tubes were correctly positioned in the omasal canal, sampling time and volumes, sample processing, isolation of fluid and particle-associate bacteria, digesta marker analyses, and preparation of omasal true digesta were performed according to Reynal and Broderick (2005) and Brito et al. (2007), except that ammonia and protozoa were not isolated for determination of ^{15}N enrichment. At each sampling

time, 325 mL of omasal sample was collected and split into 2 subsamples (125 and 200 mL). The four 125-mL subsamples were pooled and stored on ice to yield daily composite of 500 mL from each cow, which was used for bacterial isolation. The four 200-mL subsamples were pooled and stored at -20°C over the 3 d to obtain a single 2.4-L composite from each cow in each period for late separation into the 3 omasal phases.

The triple marker technique of France and Siddons (1986) was used to determine the proportions to recombine the 3 phases to produce omasal true digesta. Samples of omasal true digesta were analyzed for total N, DM (105°C), ash, OM, NDF, and ADF as described previously for feed samples in experiment 1. Samples of true digesta and isolated bacteria were treated with K_2CO_3 (Brito et al., 2007) to remove residual ammonia and analyzed for total N (equivalent to NAN) and for ^{15}N abundance using a Costech 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) interfaced to a Thermo-Finnigan Delta-Plus Advantage isotope ratio mass spectrometer (Thermo-Electron GmbH, Bremen, Germany). Flows of dietary nutrients and microbial origin and extents of ruminal digestion were calculated using the procedures described by Brito et al. (2007).

On d 27 of each period, approximately 150 mL of digesta were collected from 3 locations in the rumen at 0 (before feeding), 2, 4, 6, 8, 12, 18, and 22 h after feeding, strained through 2 layers of cheesecloth, and pH was measured immediately using a glass electrode. Two 10-mL samples of ruminal fluid were preserved in scintillation vials by addition of 0.2 mL of 50% H_2SO_4 and stored at -20°C. Before analysis, samples were thawed and centrifuged (15,300 x *g* for 20 min at 4°C) and flow-injection analyses (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI) were applied to

supernatants to determine ammonia, using a phenol-hypochlorite method (Lachat Method 18-107-06-1-A; Lachat), and total AA, using a fluorometric procedure based on the reaction with *o*-phthaldialdehyde (Roth, 1971). Leucine was the standard in the *o*-phthaldialdehyde assay and total AA are reported in Leu equivalents. Samples were also thawed and centrifuged (30,000 x *g* for 30 min at 4°C) for determination of individual and total ruminal VFA using a modification of the gas-liquid chromatography method for free fatty acids described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte, PA) with flame-ionization detection. Standards and supernatants (0.5 or 1 µL) were injected onto a ZB-FFAP capillary column (30 m x 0.53 mm x 1.0 µm; no. 7HK-G009-22; Phenomenex Inc., Torrance, CA) with helium carrier gas at 100 kPa and a flow rate of 20 mL/min. Column oven temperature was 100°C at injection; after 2 min, the temperature was increased to 130°C at a rate of 10°C/min. Injector and detector temperatures were 230°C and 250°C, respectively. This method did not resolve isovalerate and 2-methyl butyrate, which are reported as isovalerate plus 2-methylbutyrate.

Statistical Analysis

Experiment 1. Data were analyzed as a randomized complete block design with the data from the preliminary 2-week covariate period using the MIXED procedure of SAS (2003, SAS Institute Inc., Cary NC) with week of treatment as repeated measure using the first order compound symmetry (cs) covariance structure. The covariance structure of compound symmetry was selected based on best fit Akaike information criterion. The following model was used to fit the data to assess least squares means for intake and lactation performance:

$Y_{ijklm} = \mu + Cov_i + B_j + W_k + D_l + DW_{lk} + C_{m(j)} + e_{ijklm.}$

where, Y_{ijklm} = dependent variable, μ = overall mean, Cov_i = effect of covariate period i, B_j = effect of block j, W_k = effect of week k, D_l = effect of dietary treatment l, DW_{lk} = interaction between diet l and week k, $C_{m(j)}$ = effect of cow m (within block j) and $e_{ijklm.}$ = residual error. All terms were considered fixed, except for $C_{m(j)}$ and e_{ijklm} which were considered random. Degrees of freedom were calculated using the between-within option. Orthogonal contrasts were used to compare the effects of different protein supplements: SBM vs. CM + TCM and CM vs. TCM. For apparent total tract digestibility and excretion, the same model was used except that the covariate period was removed from the model. Statistical differences were declared at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$.

Experiment 2. Statistical analyses were performed as a replicate 3 × 3 Latin square design using the MIXED procedure of SAS (2003, SAS Institute Inc., Cary NC).

One cow injured her foot during the third feeding period and was dropped from the study. The results of the first two feeding periods of the injured cow were included in the statistical analysis. The following model was used to fit the data to assess effects of dietary treatments:

$Y_{ijkl} = \mu + S_i + P_j + C(S)_{kj} + T_l + ST_{il} + e_{ijkl,}$

where Y_{ijk} = dependent variable; μ = overall mean; S_i = effect of square i; P_j = effect of period j; $C(S)_{kj}$ = effect of cow k (within square i); T_l = effect of treatment l; ST_{il} = interaction between square i and treatment l; and e_{ijkl} = residual error. The following model was used for ruminal variables, for which repeated measurements over time were used (pH, ammonia, total free AA, and individual and total VFA):

$Y_{ijklm} = \mu + S_i + P_j + C(S)_{kj} + T_l + ST_{il} + Z_m + ZT_{ml} + e_{ijklm,}$

where Y_{ijkl} = dependent variable; μ = overall mean; S_i = effect of square i; P_j = effect of period j; $C(S)_{kj}$ = effect of cow k (within square i); T_l = effect of treatment l; ST_{il} = interaction between square i and treatment l; Z_m = effect of time m; ZT_{ml} = interaction between time m and treatment l; and e_{ijklm} = residual error. All terms were considered fixed, except $C(S)_{kj}$ and e_{ijkl} which were considered random. The covariance structure autoregressive (AR(1)) was selected based on best fit Akaike information criterion. Degrees of freedom were calculated using the Kenward-Rogers option. Orthogonal contrasts were used to compare the effects of different protein supplements: SBM vs. CM + TCM and CM vs. TCM. Statistical differences were declared at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Diet did not statistically affect ($P > 0.05$) DMI and feed efficiency (Table 3). In a meta-analysis, Martineau et al. (2013) observed a positive response of 0.24 kg/d in DMI for a CM diet compared to different protein supplements. In the present study, we observed similar numerical results, despite no statistical difference in DMI, inclusion of both CM at 11% in the TMR numerically increased DMI by 0.2 kg/d compared to cows fed SBM. Similar to our results, Shingfield et at. (2003) did not find differences in DMI between heat-treated rapeseed meal expeller and SBM for dairy cows fed grass silage based diets.

A diet effect was not observed ($P > 0.05$) on yield of milk 3.5% FCM, and ECM (Table 3). Despite no statistically significant effects of diet, yield of milk, 3.5% FCM, and ECM, for cows fed both CM increased on average 0.9, 1.0, and 0.9 kg/d, respectively, compared with SBM diet. Two meta-analyses evaluating the effects of diets with CM versus SBM or other protein supplements reported an increase of milk yield for CM diets (Huhtanen et al., 2011; Martineau et al., 2013). Furthermore, Broderick et al. (2015) found an increase in milk yield (0.9 kg/d), and ECM (0.4 kg/d) when replacing SBM with CM. Since we observed similar or greater numerical differences for the same variables compared to literature, it is likely that our study lacked the statistical power needed to detect a significant difference in milk yield.

In the present study, cows fed the CM diet had an average numerical increase of 0.8 kg of milk/d when compared to cows fed the TCM diet. Extrusion plus molasses was used to treat CM, and, based on our RUP flow at the omasal canal results, the TCM diet was not effective in increasing RUP flow compared to CM diet (Table 6). It is well known that extrusion may be an effective method to increase the RUP content of protein supplements (Solanas et al., 2005). However, in some cases it may not increase RUP or it may decrease AA availability due to overheating (Mustafa et al., 2003; Deacon et al., 1988). We speculate that the extrusion process was not effective in increasing the RUP content of CM due to the reduction of true protein soluble fraction in CM during the heating process. As shown in previous studies the soluble protein fraction of CM may escape ruminal degradation, thus contributing to the RUP fraction of CM (Hedqvist and Udén, 2006; Bach et al., 2008). Furthermore, Ahvenjärvi et al. (1999) found a decreased in intake and omasal flow of true protein soluble fraction and no difference in omasal

flows of neutral detergent-soluble protein and neutral detergent-insoluble protein fractions for cows fed a diet with heat-moisture rapeseed cake compared with rapeseed meal. Therefore, the extrusion process of CM may decrease the soluble content of CM that would escape ruminal degradation and, thus, RUP flow. This may explain why the extrusion process in the present study did not increase the RUP content of TCM diet. In addition, it is likely that in the present study the extrusion process decreased apparent total tract digestibility of nutrients (DM, OM, CP, and NDF), since there was a tendency ($P = 0.10$) for total tract digestibility of those nutrients to be lower for TCM diet compared to CM (Table 4). Furthermore, according to Newkirk et al. (2003), processing at elevated temperatures may decrease the AA bioavailability, which may explain the numerical differences in milk yield between CM diets.

Diet did not affect ($P > 0.05$) milk fat, milk true protein, and SNF yields and concentrations (Table 3). However, both CM diets increased ($P = 0.03$) milk lactose concentration. Despite this increase in lactose concentration, the magnitude of this difference does not seem to be biologically relevant.

Both CM diets decreased ($P < 0.01$) MUN by 8% compared to SBM diet (Table 3) potentially indicating an improvement in N utilization for CM diets. The reduction in MUN in cows fed CM diets may be related to the increased concentration of plasma branched-chain AA (**BCAA**). Appuhamy et al. (2011) reported that infusion of 60 g/d of BCAA did not increase milk protein efficiency, but decreased MUN. Therefore, they speculated that infusion of BCAA may promote the synthesis of other body tissue proteins rather than an increase in AA catabolism. In addition, Martineau et al. (2014) reported a reduction in MUN, and an increase in plasma concentration of BCAA for cows

fed CM compared to other protein supplements. Shingfield et al. (2003) also found a reduction in blood urea N (BUN), and an increase in plasma His, essential AA and BCAA concentrations in cows fed either heat-treated rapeseed expeller or SBM diets. Moreover, both CM diets decreased ($P < 0.01$) urinary urea-N (% of total urinary N), 68.8 vs. 72.2 %, and fecal-N (% of total N intake) 29.7 vs. 31.6 %, for CM diets and SBM, respectively (Table 4). In agreement with our results, Broderick et al. (2015) observed a significant decrease in MUN and excretion of urinary urea-N for cows fed CM compared with SBM. The reduction in urinary urea-N may indicate a potential to reduce environmental impact for cows fed CM diet compared to SBM.

Diet did not affect ($P > 0.05$) urinary volume, urinary excretion of allantoin, uric acid, total PD derivatives, and estimated microbial N flow (Table 4). The average across diets values observed in the present study were 29.1 L/d and 507, 60, and 566 mmol/d for urine volume and allantoin, uric acid, and total PD excretion, respectively. Our data are within the range of values observed in previous studies for those variables (Reynal et al., 2005; Colmenero and Broderick, 2006). The dietary average of microbial N flow estimated using PD as microbial marker in the present study was 382 g/d, which was overestimated by 21% compared to microbial NAN flow (g/d) measured using ^{15}N as a microbial marker. Our results are in agreement with previous results reported in the literature, where Reynal et al. (2005) found 22% overestimation and Faciola and Broderick (2014) found 28% overestimation of microbial N flow when comparing these microbial markers. Despite these differences, Reynal et al. (2005), reported that using PD in spot urine samples as a microbial marker was as effective as ^{15}N for detecting treatment differences in microbial N flow, which agrees with the results of the present

study, where no dietary effects in microbial N flow were observed using either markers (PD or ^{15}N).

Diets affected ($P < 0.01$) apparent total tract digestibility of DM, OM, CP, and NDF (Table 4). Mean apparent total tract digestibility for the CM diets were 1.9, 1.7, 3.4, and 3.0 % units greater than that for the SBM diet for, respectively, DM, OM, CP, and NDF. The increase in DM and OM digestibility may be explained by the greater digestibility of CP and NDF, which are substantial components of OM. In agreement with our results, Brito and Broderick (2007) found an increase in NDF digestibility for cows fed CM diet compared to SBM. The increase in CP digestibility is in line with our omasal data, where we observed a tendency ($P < 0.10$) to increase the amount of N truly digested in the rumen and RDP supply at the omasal canal for both CM diets compared to SBM (Table 6).

Experiment 2

Experiment 2 aimed to evaluate omasal nutrient flow and ruminal fermentation pattern of the same dietary treatments used in Experiment 1 (SBM, CM, and TCM). Diets did not affect ($P > 0.05$) DMI, nutrient flow at the omasal canal, or ruminal digestibility of OM, NDF, and ADF (Table 5). Agreeing with our results, Paula et al. (2017) did not observe differences in ruminal digestibility of OM, NDF, and ADF between diets containing CM with different RUP content, and between CM diets vs. SBM in an in vitro study. Brito et al. (2007) found similar values for OM truly digested in the rumen (**OMTDR**) for diets containing SBM (65.5% of OM intake) or CM (63.3% of OM intake). Furthermore, Chibisa et al. (2012) found 62.7% for OMTDR for diet with inclusion of 8% of CM. In the present study, overall OMTDR was 90% of OM total tract

digestibility, 63.4% for ruminal (Table 5), and 70.3 % for total tract digestibility (Table 4). Therefore, our data are in line with the expected values for ruminal and total tract OM digestibility.

The average across diets for ruminal NDF digestibility in the present study was 31.4%. Ruminal NDF digestibility was 67% of total tract NDF digestibility. Ruminal NDF digestibility observed in our study were lower than the values reported in other study in which TCM was used as protein supplement for dairy cows (Krizsan et al., 2017). However, they were similar to values observed in high producing cows using omasal sampling (Faciola and Broderick, 2014) who found that ruminal NDF digestibility was 63% of total tract NDF digestibility, factors that may explain these differences include DMI, rate of passage, and milk yield.

Diet did not affect ($P > 0.05$) estimated non-microbial NAN (**NMNAN**) flow, total microbial NAN flow, and efficiency of microbial protein synthesis. However, an increase was observed ($P = 0.04$) in dietary N intake, RDP supply ($P = 0.01$), and a tendency ($P = 0.10$) for increased N truly digested in the rumen when both CM diets were fed compared with SBM diet (Table 6). Cows fed CM had a numerically greater DMI (0.9 kg) compared to cows fed SBM, which explained the greater N intake for cows fed CM; consequently, more N was available to the rumen microorganisms to degrade, which may explain the increase of N truly digested in the rumen, and the greater RDP supply for both CM diets. Studies comparing ruminal CP degradability of CM with other protein supplements have reported inconsistent results. Paz et al. (2014) found greater ruminal CP degradability for CM compared to SBM (75.5 vs. 68.8% of CP, respectively), whereas Maxin et al. (2013b) observed lower ruminal CP degradability for CM compared

SBM (47.5 vs. 58.5% of CP, respectively). Both studies estimated ruminal CP degradability of these protein supplements as a sole ingredient using in situ methodology. Previous studies comparing the effect of CM and SBM in the total diet (Paula et al., 2017; Rinne et al., 2015) did not observe difference in ruminal N metabolism. The inconsistency between the present study and these previous studies may be due to a DMI effect since, in both studies the DMI were not different.

We hypothesized that treating CM by extrusion would improve the flow of RUP at the omasal canal compared to regular CM. However, our results did not support this hypothesis; treating CM by extrusion did not affect the RUP flow for TCM compared to CM. Therefore, our results indicate that the extrusion process applied to CM was not effective in enhancing RUP flow. As mentioned earlier, to our knowledge there are no data in the literature reporting RUP flow when TCM was fed as the main protein supplement for lactating dairy cows fed corn and/or alfalfa silage-based diets. Ahvenjärvi et al. (1999) did not observe difference between rapeseed meal and heat-moisture-treated rapeseed cake on dietary NAN and microbial NAN flow at the omasal canal for cows fed a grass silage and barley based diet. Recently, Kriszan et al. (2017) observed a linear increase in NANMN and a tendency to decrease bacterial NAN flow and bacterial efficiency when incremental amounts of TCM were added to grass silage-based diets. As reported in previous studies, increased flow of NANMN to the small intestine is often related to a decrease in microbial NAN flow (Ipharraguerre and Clark 2005; Santos et al., 1998). In the present study, we did not observe differences in microbial NAN and NANMN flows at the omasal, possibly due to the ineffectiveness of the extrusion process in increasing the RUP content in CM as earlier discussed.

However, there are some studies comparing regular CM with heat-treated CM or CM treated with heat and lignosulfonate on performance of lactating dairy cows (Rinne et al., 1999; Wright et al., 2005; Mutsvangwa et al., 2016). In these studies, the authors did not find greater animal performance when only heat-treatment was applied to the CM compared to regular CM. However, Wright et al. (2005) observed an increase in the performance of cows when both heat and lignosulfonate were applied to regular CM. In addition, von Keyserlingk et al. (2000) using in situ methodology, reported a decrease in ruminal and an increase in intestinal digestibility for the canola treated with heat plus lignosulfonate compared to untreated and heat-treated canola. It is likely that the process used to treat canola meal in the present study was not effective to enhance the RUP flow to the small intestine, which may explain the lack of response to TCM.

The averages across diets in the present study for NMNAN and total microbial NAN flow were 190 and 484 g/d, respectively. When expressed as a proportion of NAN intake, the dietary average values were 28 and 72% for NMNAN and total microbial NAN, respectively. These values are within the range values observed in previous studies with lactating cows, which ranged from 28 to 33% for NMNAN (% of NAN intake), and 67 to 72% for total microbial NAN (% of NAN intake) (Brito et al., 2007; Chibisa et al., 2012; Faciola and Broderick, 2014).

Diets did affect ($P = 0.02$) ruminal ammonia N (Table 7). Both CM diets had greater ruminal NH_3-N concentration compared to SBM, 6.68 mg/dL (mean of both CM diets) vs. 5.91 mg/dL for SBM. No differences ($P = 0.69$) were observed between the two CM diets. According to Reynolds and Kristensen (2008), ruminal NH_3-N is positively correlated with N intake and, as previously discussed, cows fed both CM diets had a

greater N intake, which may explain the greater ruminal NH_3-N concentration for CM diets. Diet did not affect ($P > 0.05$) ruminal concentration of free AA, total VFA, total branched-chain VFA (m*M*), molar proportions of acetate, propionate, butyrate, valerate, and isobutyrate, and pH (Table 7). Agreeing with our results, Brito and Broderick. (2007) comparing diets with SBM or CM for lactating dairy cows did not find differences in total VFA, acetate, butyrate, isobutyrate, valerate. However, we observed an increase ($P = 0.03$) in the molar proportion of isovalerate when TCM was fed compared to CM. Conversely, Ahvenjärvi et al. (1999) reported lower molar proportion of isovalerate for heat-moisture-treated rapeseed cake diet compared to rapeseed meal. The inconsistency between these studies may be due to the type of canola used (heat-treated rapeseed cake vs. heat-treated CM) or due to the different heating processes used to treat the protein supplements.

CONCLUSIONS

Our results indicate that under the conditions of the present study, treating CM by extrusion was not effective in improving CM utilization by lactating in dairy cows. Diets did not affect performance of dairy cows. However, when compared to the SBM diet, both CM diets increased digestibility and N intake, and tended to increase RDP supply at the omasal canal and N truly digested in the rumen, and to decrease MUN and N excretion in feces and urine. Therefore, CM may reduce the environmental impact compared to SBM, due to a lower urea N excretion as a proportion of total urinary N.

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Table 1. Chemical composition of major dietary ingredients (% DM, unless otherwise stated)[1]

	AS		CS		HMSC		SBM		CM		TCM	
Item	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DM, % as fed	54.6	0.7	40.7	0.3	74.4	0.6	90.0	0.2	92.0	0.8	91.3	0.4
OM	90.2	0.1	96.6	0.1	98.3	0.1	93.3	0.1	92.3	0.1	92.3	0.2
CP	22.6	0.2	6.1	0.0	7.5	0.1	53.6	0.5	41.8	0.3	40.2	0.1
NDF	39.1	0.4	35.0	0.5	7.1	0.2	7.8	0.3	28.9	0.2	29.1	0.3
ADF	29.9	0.4	18.4	0.3	1.5	0.1	4.6	0.2	18.6	0.2	19.3	0.2
NPN, % of total N	47.2	0.8	52.8	1.2	22.9	2.6	-	-	-	-	-	-
NH_3, % of total N	4.6	0.1	7.4	0.5	1.4	0.3	-	-	-	-	-	-
Peptides, % of total N	15.6	0.4	12.8	0.4	-	-	-	-	-	-	-	-
Total AA-N, % of total N	27.0	0.4	32.6	0.8	-	-	-	-	-	-	-	-
pH	4.7	0.02	3.79	0.02	4.56	0.06	-	-	-	-	-	-

[1]AS = alfalfa silage, CS = corn silage, HMSC = high-moisture shelled corn, SBM = solvent soybean meal, CM = canola meal, TCM = treated CM

Table 2. Dietary ingredients and chemical composition (% DM, unless otherwise stated)

	Diets[1]		
Item	SBM	CM	TCM
Alfalfa silage	31.3	31.2	31.3
Corn silage	28.9	28.8	28.8
High moisture shelled corn	25.1	22.3	22.4
Solvent soybean meal	8.4	-	-
Canola meal	-	11.2	-
Treated canola meal	-	-	11.4
Soy hulls	3.93	3.92	3.93
Vitamin and Minerals premix[2]	2.45	2.48	2.48
Chemical composition			
DM, %	55.8	55.9	55.4
OM	92.3	92.1	92.3
NDF	27.1	29.5	29.6
ADF	17.2	18.8	18.9
CP	15.7	15.7	15.6
RDP[3]	10.2	11.1	11.1
RUP[4]	5.2	5.0	4.8
NFC[5]	44.5	42.8	39.6
Fat[6]	2.88	2.96	2.96
NEL[7], Mcal/kg of DM	1.55	1.54	1.57

[1]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.
[2]Provided (per kilogram of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, 16 IU of vitamin E, and 12 mg of monensin.
[3]RDP was calculated from experiment 2 as RDP, % of DM = (total CP intake, kg/d – omasal RUP flow, kg/d) x 100/DM intake, kg/d.
[4]RUP was calculated from experiment 2 as RUP, % of DM = (total omasal CP flow, kg/d – omasal microbial CP flow, kg/d) x 100/DM intake, kg/d.
[5]NFC = 100 – (%NDF + %CP + %Fat + %ash) according to the NRC (2001) model
[6]Fat contents of individual dietary ingredients were used from the NRC (2001) nutrient composition tables.
[7]NE_L = Net energy lactation, estimated using the NRC (2001) model.

Table 3. Effect of different protein supplements on dry matter intake, milk production and composition in lactating dairy cows[1]

	Diet[2]				Contrasts *P* -value[3]	
Item	SBM	CM	TCM	SEM	SBM vs. CM+TCM	CM vs. TCM
DMI, kg/d	26.7	27.1	26.7	0.74	0.81	0.75
Yield						
Milk, kg/d	40.0	41.3	40.5	1.01	0.48	0.62
3.5% FCM, kg/d[4]	42.9	44.1	43.6	1.10	0.49	0.76
ECM, kg/d[5]	39.2	40.1	40.1	1.02	0.45	0.97
Feed conversion						
Milk/DMI	1.51	1.53	1.52	0.04	0.80	0.79
ECM/DMI	1.49	1.49	1.51	0.04	0.83	0.80
Milk component						
Milk fat, %	4.07	4.09	4.11	0.08	0.80	0.88
Milk fat, kg/d	1.59	1.64	1.62	0.04	0.42	0.67
Milk true protein, %	3.20	3.14	3.17	0.03	0.18	0.51
Milk true protein, kg/d	1.25	1.25	1.28	0.03	0.70	0.53
Milk lactose, %	4.83	4.86	4.89	0.02	0.03	0.18
Milk lactose, kg/d	1.91	1.94	1.98	0.05	0.47	0.62
SNF, %	8.91	8.93	8.95	0.04	0.61	0.72
SNF, kg/d	3.52	3.57	3.59	0.09	0.63	0.90
MUN, mg/dL	13.7	12.8	12.5	0.25	<0.01	0.43
BW gain, kg/d	0.52	0.38	0.59	0.06	0.67	0.03

[1]Data from the 45 lactating cows.

[2]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.

[3]Orthogonal contrasts for the effects of different protein supplement (SBM vs. CM + TCM), and (CM vs. TCM).

[4]FCM = 0.4318 × milk yield + 16.23 × fat yield (Sklan et al., 1992).

[5]ECM = milk yield, kg/d x [(0.0929 x percentage of fat) + (0.0563 x percentage of true protein) + (0.0395 x percentage of lactose)] / 0.749 (Krause and Combs, 2003).

Table 4. Effect of different protein supplements on nitrogen excretion and apparent digestibility in lactating dairy cows[1]

	Diet[2]				Contrasts *P* -value[3]	
Item	SBM	CM	TCM	SEM	SBM vs. CM+TCM	CM vs. TCM
N intake, g/d	653	692	692	22.7	0.17	1.00
Milk protein N, g/d	198	200	207	5.8	0.43	0.40
Milk protein N, % of N intake	30.7	29.2	30.1	0.87	0.32	0.48
Urinary excretion						
Urine volume, L/d[4]	28.4	30.2	28.8	1.44	0.54	0.50
Total N, g/d	219	221	216	7.3	0.92	0.57
Total N, % of N intake	34.0	32.5	31.5	1.01	0.12	0.46
Urea N, g/d	157	152	147	5.0	0.22	0.44
Urea N, % of total urinary N	72.2	69.2	68.4	0.90	<0.01	0.52
Allantoin, mmol/d	495	499	526	17.2	0.41	0.28
Uric acid, mmol/d	63.6	57.4	59.0	3.46	0.22	0.75
Purine derivatives, mmol/d[5]	558	556	585	19.7	0.62	0.31
Microbial N flow, g/d[6]	376	382	388	14.5	0.62	0.78
Fecal N excretion						
N, g/d	207	207	210	8.0	0.96	0.47
N, % of intake	31.6	29.2	30.3	0.45	<0.01	0.08
Apparent digestibility, %						
DM	68.4	70.9	69.7	0.44	<0.01	0.08
OM	70.2	72.4	71.3	0.42	<0.01	0.07
CP	64.7	68.9	67.3	0.68	<0.01	0.09
NDF	45.1	49.1	47.1	0.80	<0.01	0.08

[1]Data from the 45 lactating cows.
[2]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.
[3]Orthogonal contrasts for the effects of different protein supplement (SBM vs. CM + TCM), and (CM vs. TCM).
[4]Estimated from creatinine concentration in spot urine samples assuming an excretion of 29 mg of creatinine/
kg of BW (Valadares et al., 1999).
[5]Allantoin plus uric acid.
[6]Estimated from urinary excretion of purine derivatives according to Valadares et al. (1999).

Table 5. Effect of different protein supplements on intake, flow at omasal the canal, and ruminal digestibility of DM, OM, NDF, and ADF in lactating dairy cows[1]

	Diet[2]				Contrasts *P* -value[3]	
Item	SBM	CM	TCM	SEM	SBM vs. CM+TCM	CM vs. TCM
DM						
Intake, kg/d	25.4	26.0	26.7	1.62	0.28	0.48
Flow at the omasal canal, kg/d	18.9	19.3	19.8	1.33	0.34	0.52
Apparently digested in the rumen, kg/d	6.42	6.70	6.90	0.53	0.34	0.69
% of DMI	25.3	25.9	25.8	1.62	0.59	0.94
OM						
Intake, kg/d	23.5	23.9	24.5	1.48	0.36	0.53
Flow at the omasal canal, kg/d	14.2	14.4	15.3	0.91	0.25	0.23
Apparently digested in the rumen, kg/d	9.48	9.62	9.28	0.89	0.92	0.72
% of OM intake	39.8	40.1	37.4	2.24	0.66	0.37
Truly digested in the rumen, kg/d	15.1	15.5	15.1	1.12	0.81	0.72
% of OM intake	64.3	64.4	61.6	1.82	0.46	0.22
NDF						
Intake, kg/d	7.63	7.71	8.30	0.54	0.33	0.76
Flow at the omasal canal, kg/d	5.4	5.01	5.75	0.51	0.97	0.28
Apparently digested in the rumen, kg/d	2.21	2.70	2.55	0.62	0.49	0.84
% of NDF intake	29.5	34.8	30.1	6.01	0.62	0.55
ADF						
Intake, kg/d	5.06	4.97	5.48	0.36	0.50	0.16
Flow at the omasal canal, kg/d	3.36	3.12	3.63	0.32	0.96	0.25
Apparently digested in the rumen, kg/d	1.70	1.82	1.83	0.43	0.75	0.98
% of ADF intake	34.0	36.5	32.7	5.94	0.62	0.91

[1]Data from the 6 ruminally cannulated cows.
[2]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.
[3]Orthogonal contrasts for the effects of different protein supplement (SBM vs. CM + TCM), and (CM vs. TCM).

Table 6. Effect of different protein supplements on intake and flow of N fractions at the omasal canal in lactating dairy cows[1]

	Diet[2]				Contrasts *P* -value[3]	
Item4	SBM	CM	TCM	SEM	SBM vs. CM+TCM	CM vs. TCM
Dietary N intake, g/d	625	668	679	39	0.04	0.64
Omasal flows						
Total NAN, g/d	669	688	671	50	0.82	0.76
NAN % of N intake	107	101	99	4.3	0.16	0.76
N truly digested in the rumen, g/d	413	472	471	29	0.10	0.97
RDP supply						
kg/d	2.58	2.96	2.95	0.18	0.10	0.81
% of diet CP	66.1	69.0	69.9	3.5	0.40	0.85
% of DMI	10.2	11.1	11.1	0.57	0.16	0.95
RUP flow						
kg/d	1.33	1.28	1.30	0.18	0.78	0.92
% of diet CP	33.9	31.0	30.1	3.5	0.41	0.85
% of DMI	5.2	5.0	4.8	0.52	0.56	0.81
NMNAN[4] flow						
g/d	187	200	183	21.1	0.72	0.31
% of total NAN	27.9	29.7	27.2	2.49	0.79	0.37
% of N intake	29.5	29.9	27.2	2.44	0.54	0.21
Microbial NAN flows						
FAB-NAN[4]						
g/d	185	176	190	7.89	0.82	0.20
% of microbial-NAN	38.7	37.2	38.9	2.36	0.78	0.56
PAB-NAN[4]						
g/d	298	306	298	35.1	0.89	0.84
% of microbial-NAN	61.3	62.8	61.1	2.36	0.78	0.56
Total microbial NAN						
g/d	482	482	488	40.7	0.94	0.90
% of total NAN	72.1	70.3	72.7	2.49	0.79	0.37
Microbial efficiency, g of NAN/kg of OMTDR[4]	32.2	30.5	32.8	1.95	0.81	0.38

[1]Data from the 6 ruminally cannulated cows.

[2]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.

[3]Orthogonal contrasts for the effects of different protein supplement (SBM vs. CM + TCM), and (CM vs. TCM)

[4]NMNAN = non-microbial NAN; FAB – and PAB-NAN = fluid- and particle-associated bacterial NAN; OMTDR = OM truly digested in the rumen.

Table 7. Effect of different protein supplements on pH and metabolite concentrations[1]

Item	Diet[2]			SEM	Contrasts *P* -value[3]	
	SBM	CM	TCM		SBM vs. CM+TCM	CM vs. TCM
pH	6.44	6.46	6.51	0.089	0.43	0.53
NH_3-N, mg/dL	5.91	6.60	6.76	0.335	0.02	0.69
Total free AA, m*M*	2.56	3.08	2.68	0.327	0.30	0.29
Total VFA, m*M*	77.0	79.5	76.5	3.53	0.74	0.42
Acetate, % of total VFA	66.3	66.8	67.1	1.13	0.16	0.53
Propionate, % of total VFA	19.9	20.3	19.3	0.98	0.84	0.13
Acetate:propionate	3.40	3.38	3.52	0.203	0.58	0.19
Butyrate, % of total VFA	10.4	9.71	10.2	0.362	0.28	0.37
Valerate, % of total VFA	1.10	1.07	1.07	0.096	0.50	0.91
Isovalerate + 2-methyl butyrate, % of total VFA	1.49	1.36	1.53	0.066	0.45	0.03
Isobutyrate, % of total VFA	0.84	0.79	0.85	0.041	0.51	0.17
Branched-chain VFA, m*M*	1.81	1.71	1.82	0.085	0.63	0.27

[1]Data from the 6 ruminally cannulated cows.

[2]SBM = dietary treatment with solvent soybean meal as protein supplement; CM = dietary treatment with canola meal as protein supplement; TCM = dietary treatment with treated canola meal as protein supplement.

[3]Orthogonal contrasts for the effects of different protein supplement (SBM vs. CM + TCM), and (CM vs. TCM).

Chapter 4: In preparation to be submitted to Journal of Dairy Science

Assessing potentially digestible NDF and energy content of canola meal from twelve Canadian crushing plants over four production years

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ABSTRACT: The objective of this study was to assess NDF digestibility and energy content of canola meals (CM) produced in Canada over a 4-year period. Canola meal samples were collected from 12 Canadian crushing plants over 4-years (2011-2014, total = 48) and analyzed for chemical composition, potentially digestible NDF (pdNDF), total digestible nutrients at maintenance (TDN_{1x}), and NEL simulating a cow consuming 3x maintenance (NEL_{3x}). To estimate TDN_{1x} and NEL_{3x}, pdNDF was calculated as: 1) $pdNDF_{OBS}$ = (NDF - NDICP - iNDF), using observed CM iNDF values after 288-h in situ ruminal incubations; 2) $pdNDF_{NRC}$ = (NDF - NDICP - ADL) x {1- [ADL/(NDF-NDICP)]$^{0.667}$}, according to NRC 2001; 3) $pdNDF_{CNCPS}$ = (NDF - NDICP - iNDF), according to the CNCPS that calculates iNDF as acid detergent lignin (ADL) × 2.4. Concentrations of NDF, NDICP, and ADL in all equations were given in % of DM. Truly digestible NDF was estimated multiplying the observed and predicted pdNDF by 0.75. Then TDN_{1x} and NEL_{3x} were calculated assuming a diet with 74% of TDN_{1x} according to NRC 2001 equations. Regressions of predicted (NRC or CNCPS) vs. observed values were performed using Proc Reg of SAS (Table 1). Potentially digestible NDF_{OBS}, $pdNDF_{NRC}$, and $pdNDF_{CNCPS}$ averaged 15, 8, and 2.4% of DM, respectively. The TDN_{1x} averages were 73, 67, and 64%, respectively. The NEL_{3x} averages were 1.88, 1.73, and 1.63 Mcal/kg, and ranged from 1.73 to 2.08; 1.51 to 1.94; and 1.4 to 1.87 Mcal/kg DM for NEL_{3xOBS}, NEL_{3xNRC}, and $NEL_{3xCNCPS}$, respectively. Our results indicate that NEL_{3x} from CM diets may be underestimated in current nutritional models due to underestimations in CM NDF digestibility. More accurate information on CM NDF digestibility may improve energy content estimation, thus improving diet formulation accuracy.

Key words: acid detergent lignin, indigestible NDF, in situ

INTRODUCTION

Canola meal (CM) has a complex carbohydrate matrix. The fiber is mainly composed of acid detergent fiber (ADF), usually neutral detergent fiber (NDF) levels in CM is approximately 10% greater than ADF.The fiber content of CM generally is greater than other vegetables proteins, because the canola seed has a relatively high amount of hull, which stays with the meal after oil extraction and meal processing consequently the energy content of CM is lower than soybean meal (SBM) (Newkirk, 2011). However, there have been speculations about the possibility of inaccuracies in the current values for canola meal (**CM**) indigestible NDF (**iNDF**), and NDF digestibility, possibly due to the high lignin content of CM. Current prediction models such as the NRC (2001) and the Cornell Carbohydrate and Protein system (**CNCPS**; Fox et al., 2004) use a factorial approach to calculate the energy value of CM based on the unavailable energy in the cell wall. For instance, NRC (2001) estimates that 65% of the total CM NDF is unavailable for digestion (Canola Meal Feeding Guide, 2015). On the other hand, Cotanch et al. (2014) evaluating the iNDF content of CM using an in vitro approach observed a iNDF content of CM of 32%. Therefore, using the iNDF values estimated by NRC (2001) may underestimate the calculated metabolizable energy (**EM**), and net energy (**NE**). In addition, the low fiber digestibility values appear to not match up with the reported milk yield response when CM is included in the diet of lactating dairy cows. For example, two recent meta-analysis have shown an increase in milk yield response for cows fed diet with inclusion of CM when compared to SBM or other commonly protein supplements (Huhtanen et al., 2011; Martineau et al., 2013). Therefore, we hypothesized that iNDF and NDF digestibility are not accurately estimated based on the current prediction

models. We aimed to assess CM potentially digestible NDF and energy content in a large sample set of CM samples from 12 Canadian crushing plants harvest over 4-years (2011, 2012, 2013, and 2014; n = 48).

MATERIAL AND METHODS

Canola meal samples were collected and analyzed for chemical composition of DM (method 934.01), ash and OM (method 938.08), and ether extract (EE) (method 920.85) according to AOAC (1990), and total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI), according to AOAC (2005) (method 990.13). For NDF, samples were analyzed, being treated with thermo-stable α-amylase, and sodium sulfite according to Mertens (2002) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY). For ADF and acid detergent-insoluble CP (ADICP), samples were sequentially analyzed according to Van Soest and McQueen (1973) and adapted for the Ankom^{200} Fiber Analyzer. The nitrogen analysis in the ADF residue, was conducted using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Nitrogen in the digesta was determined by semi-automated colorimetry (Hambleton, 1977). Neutral detergent-insoluble CP (NDICP) was isolated by gravimetric determination using thermo-stable α-amylase and sodium sulfite followed by CP analysis (method 990.13; AOAC, 2005). Acid detergent lignin (**ADL**) (method 9 in a Ankom Daisy^{II} Incubator). Nonfiber carbohydrates (NFC) concentration of the feed ingredients was calculated using the equation: NFC = 100 - (%NDF + %CP + %ether extract + %ash) + NDICP, according to NRC (2001). For indigestible NDF (iNDF), approximately 1.25 g of sample was weighted, in triplicate, into Dracon bags (R510, 5 cm x 10 cm, 50 µm porosity, ANKOM® Technology), consisting of an area ratio: surface

of 20 mg/cm^2/ sample. Bags were incubated in the ventral rumen of 3 cannulated steers (average BW = 550 kg) and removed after 288 h. Before the incubations began a 14-d diet adaptation was performed. The steers were fed (DM basis) at the maintenance level diet 80:20 forage:concentrate ratio 80% alfalfa hay, 17.5% cracked corn, and 2.5% mineral premix. Animal care and handling was approved by the University of Nevada, Reno Institutional Animal Care and Use Committee (protocol no. 00588).

To estimate TDN_{1x} and NEL_{3x}, pdNDF was calculated as: 1) $pdNDF_{OBS}$ = (NDF - NDICP - iNDF), using observed CM iNDF values after 288-h in situ ruminal incubations; 2) $pdNDF_{NRC}$ = (NDF - NDICP - ADL) × {1- [ADL / (NDF - NDICP)]$^{0.667}$}, according to NRC 2001; 3) $pdNDF_{CNCPS}$ = (NDF - NDICP - iNDF), according to the CNCPS that calculates iNDF as acid detergent lignin (ADL) × 2.4, NDICP, NDF and ADL are given in % of DM. Truly digestible CP, NDF, and EE was estimated multiplying the observed and predicted pdNDF according to NRC (2001). Then TDN_{1x} and NEL_{3x} were calculated assuming a diet with 74% of TDN_{1x} according to NRC (2001) equations. Regressions of predicted (NRC or CNCPS) vs. observed values were performed using Proc Reg of SAS.

RESULTS AND DISCUSSION

Chemical composition of the CM used in the present study is shown in Table 1. Concentrations of DM, OM, CP NDF, ADF, NFC, and EE of the CM were similar than values reported in previous studies that used CM (Broderick et al., 2016; Paula et al., 2017). Despite of the importance of the nutritive values listed in Table 1 our discussion will be focused on NDF, ADF, and ADL content of CM, because these are directly related to the main objective of the present study. For NDF content no significant difference (P = 0.51) among production year was observed in the present study.

However, for ADF content was observed a significant production year effect ($P < 0.01$). We observed lower ADF values in two production years (2012 and 2013) compared to years 2011 and 2014. Acid detergent fiber values for the CM from 2012 (20.1% of DM) and 2013 (19.5% of DM) were significantly lower than the ADF values for years 2011 (22.7% of DM) and 2014 (22.4% of DM) (Table 1). Constant NDF and lower ADF values may represent an increase in hemicellulose content in years 2012 and 2013. The increase in hemicellulose in these years was coupled with a significant decrease in lignin (ADL) content. Lignin content for years 2012 (7.9 % of DM) and 2013 (8.0 % of DM) were significantly lower than for 2011 (10.7 % of DM) and 2014 (10.2 % of DM) (Table 1). Our results show that total fiber and lignin content combined remained the same between all four years, but the proportion of hemicellulose increased while the proportion of lignin decreased in years 2012 and 2013. This decreased lignin content is beneficial because lower lignin content results in higher fiber digestibility. When comparing the NRC (2001) tabular CM values for NDF (29.8 % of DM), ADF (20.5 % of DM), and lignin (9.5 % of DM) to CM average values in the present study, it is observed that NDF (32.3 % of DM) and ADF (21.2 % of DM) values increased while ADL/lignin value decreased (9.17% of DM). The differences between the present study and NRC values may be attributed to extraction methods, since the NRC (2001) only lists mechanical extracted CM, and solvent-extracted CM is the method that was used in the present study. Another possible reason for the discrepancy in the fiber values may be due to differences in genetic differences between CM plants from 2001, when the NRC model was published, to 2014, the last productive year for CM in this study, that have accumulated as the result of selective breeding programs.

Indigestible NDF observed using in situ methodology after 288 h ruminal incubation averaged 9.3 % of DM, whereas the unavailable NDF predicted according to NRC model and CNCPS averaged 16.9 and 21.95 % of DM, respectively, across all CM samples evaluated in this study. Likewise, we did not observe a linear relationship between predicted [NRC, 2001, and CNCPS] and observed concentrations of iNDF, pdNDF, TDN, and NE_L (Table 2; Figure 1 and 2). Potentially digested NDF_{OBS}, $pdNDF_{NRC}$, and $pdNDF_{CNCPS}$ averaged 15, 8, and 2.4% of DM, respectively. The TDN_{1x} averages were 73, 67, and 64%, respectively (Figure 3). The NEL_{3x} averages were 1.88, 1.73, and 1.63 Mcal/kg, and ranged from 1.73 to 2.08; 1.51 to 1.94; and 1.4 to 1.87 Mcal/kg DM for NEL_{3xOBS}, NEL_{3xNRC}, and $NEL_{3xCNCPS}$, respectively (Figure 4). Our results are in line with previous studies that reported lower indigestible NDF or greater pdNDF compared to the values predicted using the current models. Cotanch et al. (2014) found that the lignin factor (lignin * 2.4) greatly overestimated the indigestible NDF fraction of CM and other concentrate by-products with high NDF content. Furthermore, Mustafa et al. (1997) evaluating the effects of high fiber or regular CM on total tract digestibility and milk production observed an average of NDF total tract digestibility coefficient of approximately 50% of total DM intake in diets with CM, and no differences in milk production were observed between CM diets compared to SBM. Therefore, our results indicate that the NEL_{3x} from CM diets may be underestimated in current nutritional models due to overestimations of indigestible NDF and consequently underestimations in pdNDF digestibility. Further studies are needed to give more accurate information on CM NDF digestibility to improve energy content estimation, thus improving diet formulation accuracy.

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Table 1. Chemical composition of canola meals from all 4 years (% DM, unless otherwise stated)[1]

Item	2011	2012	2013	2014	Average	SEM	*P* - value
DM	93.7[a]	93.0[a]	92.2[b]	91.5[b]	92.6	0.30	< 0.01
OM	92.5	92.7	92.5	92.3	92.5	0.23	0.74
CP	40.0[b]	40.3[b]	42.6[a]	40.2[b]	40.8	0.39	<0.01
NDF	32.9	32.2	31.7	32.4	32.3	0.55	0.51
NDFcp[2]	24.2	24.4	23.5	25.4	24.4	0.67	0.21
NDICP[3]	8.64[a]	7.83[a]	8.18[a]	6.98[b]	7.9	0.43	0.05
ADF	22.4[a]	20.1[b]	19.5[b]	22.4[a]	21.2	0.36	<0.01
Lignin	10.7[a]	7.9[b]	8.0[b]	10.2[a]	9.17	0.33	<0.01
NFC	24.9	24.4	23.0	22.7	23.8	0.73	0.14
EE	3.08	3.56	3.55	3.97	3.54	0.58	0.79

[2]NDFcp = Neutral detergent fiber expressed exclusive of residual crude protein
[3]NDICP = Neutral detergent insoluble crude protein

Table 2. Regression coefficients for the relationships between predicted [NRC, 2001, and CNCPS] and observed concentrations of pdNDF, iNDF, TDN, and NE_L[1]

					P - value		
Item	Intercept	SE	Slope	SE	slope	R^2	RMSE
$pdNDF_{NRC}$[2]	7.46	1.79	-0.01	0.12	0.91	0.00	2.00
$pdNDF_{CNCPS}$[3]	-5.61	3.61	0.54	0.24	0.03	0.10	4.00
$iNDF_{NRC}$[4]	14.4	1.72	0.27	0.18	0.14	0.05	1.96
$iNDF_{CNCPS}$[5]	17.3	3.61	0.48	0.38	0.21	0.04	4.10
TDN_{1xNRC}[6]	2.18	6.54	0.89	0.09	<.01	0.70	1.65
$TDN_{1xCNCPS}$[7]	-9.20	11.9	1.00	0.16	<.01	0.47	3.02
NEL_{3xNRC}[8]	-0.06	0.18	0.95	0.09	<.01	0.70	0.04
$NEL_{3xCNCPS}$[9]	-0.46	0.32	1.11	0.17	<.01	0.50	0.08

[1]pdNDF = potentially digestible NDF; iNDF = unavailable NDF; TDN = total digestible nutrients; NEL = net energy for lactation

[2]Relationtiships between pdNDF predicted by NRC and observed pdNDF after 288 h in situ.

[3]Relationtiships between pdNDF predicted by CNCPS and observed pdNDF after 288 h in situ.

[4]Relationtiships between iNDF predicted by NRC and observed iNDF after 288 h in situ.

[5]Relationtiships between iNDF predicted by CNCPS and observed iNDF after 288 h in situ.

[6]Relationtiships between TDN predicted by NRC and observed iNDF after 288 h in situ.

[7]Relationtiships between TDN predicted by CNCPS and observed iNDF after 288 h in situ.

[8]Relationtiships between NEL predicted by NRC and observed iNDF after 288 h in situ.

[9]Relationtiships between NEL predicted by CNCPS and observed iNDF after 288 h in situ.

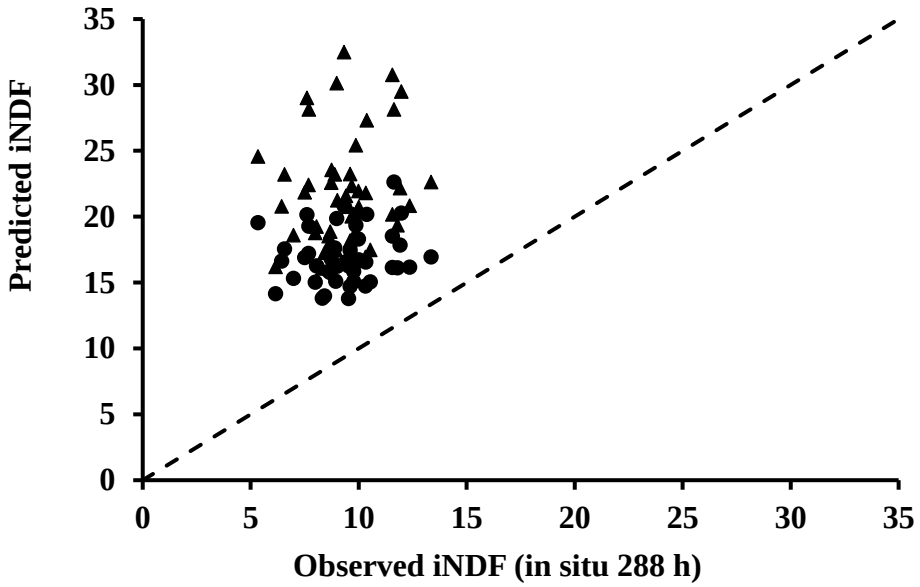

Figure 1. Relationships between predicted [NRC (2001) and Cornell Net Carbohydrate and Protein System (CNCPS)] and observed concentrations of indigestible NDF (iNDF) in experimental canola meal. Circle = NRC model; triangle = CNCPS model.

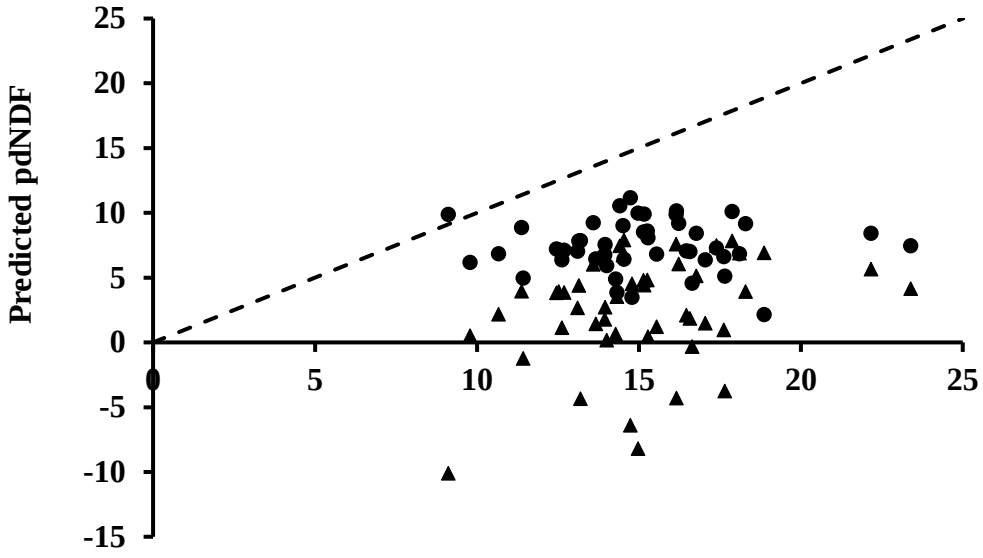

Figure 2. Relationships between predicted [NRC (2001) and Cornell Net Carbohydrate and Protein System (CNCPS)] and observed concentrations of potentially digested NDF (pdNDF) in experimental canola meal. Circle = NRC model; triangle = CNCPS model.

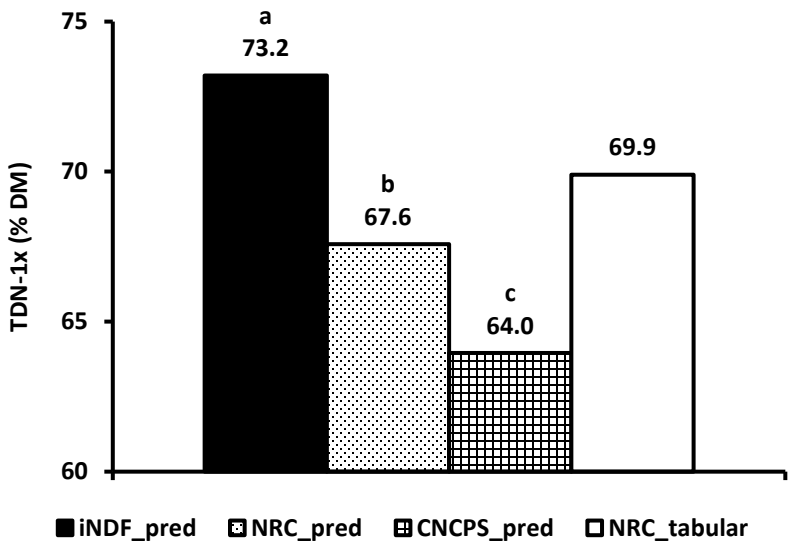

Figure 3. Estimated total digestible nutrient (TDN_{1x}) assuming a diet with 74% TDN_{1x} according to NRC (2001) based on pdNDF observed and predicted with NRC and CNCPS. [a–c]Least squares means with different superscripts differ ($P < 0.05$).

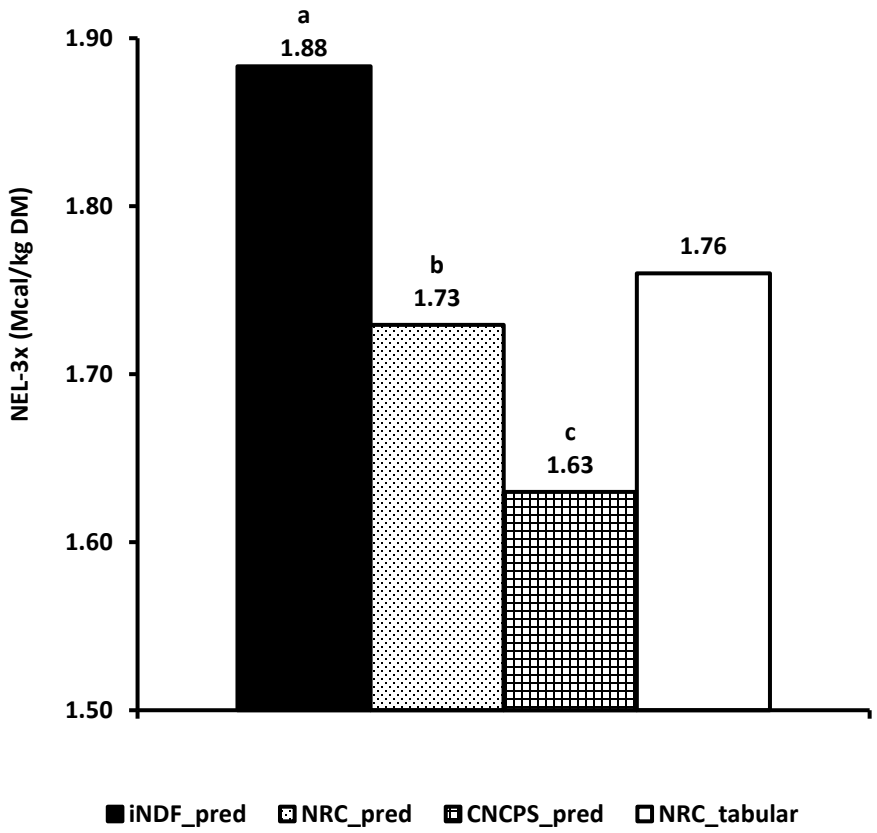

Figure 4. Estimated Net energy of lactation (NEL_{3x}) assuming a diet with 74% TDN_{1x} according to NRC (2001) based on pdNDF observed and predicted with NRC and CNCPS. [a–c]Least squares means with different superscripts differ ($P < 0.05$).

Chapter 5:

Overall Conclusions and Implications

The main objective of the present dissertation was to investigate whether the positive responses observed in milk production and nitrogen utilization efficiency in recent studies when SBM was replaced with CM as the main protein supplement in dairy cow diets, are due to a ruminal effect, a post ruminal effect, or a combination of both. Our results did not find major differences in ruminal fermentation, digestibility, and N metabolism between SBM and CM diets, which indicate that there are no major ruminal effects of replacing SBM with CM, suggesting that positive production responses previously observed when CM replaced SBM may have been due to post-ruminal effects and DMI. Other aspect that we investigated was the rumen degraded and undegraded protein content of CM. Since, most feed tables report greater metabolizable protein for SBM compared to CM due to its lower CP degradability and greater RUP content. Our results indicate that feed tables have inaccurate CM rumen undegraded and degraded protein values, and this could be due to methodological assessments of protein degradation fractions.

We also investigated whether treating CM by extrusion would increase rumen undegraded protein flow to the small intestine, nitrogen utilization and performance of dairy cows compared to regular solvent-extracted CM or SBM. However, our results indicate that treating CM by extrusion was not effective in improving CM utilization and consequently performance of lactating dairy cows. Moreover, we found that the energy content of CM may be underestimated in current nutritional models due to underestimation of potentially NDF digestibility of CM.

The implications of the present study are: 1) improvement in methodological assessments of rumen degraded and undegraded protein taking in consideration that soluble proteins, peptides, and AA may escape from ruminal degradation, thus contributing to the rumen undegraded fraction protein of CM should be pursued. 2) studies focused on post-ruminal effects of diets with CM for lactating dairy cow, for example, investigating the efficiency of utilization of essential amino acids by the mammary gland should be conducted. 3) improvement in methodological assessments to estimate the potentially digestible NDF of CM to give more accurate information on CM NDF digestibility and consequently energy content estimation should be pursued.