ASSOCIATION OF MOLECULAR STRUCTURE SPECTRAL FEATURES WITH NUTRIENT PROFILES AND AVAILABILITY AND MILK PRODUCTION PERFORMANCE OF NEWLY DEVELOPED BLEND-PELLETED PRODUCTS IN HIGH PRODUCING DAIRY COWS

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By

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ABSTRACT

The main objectives of this study were to: (1) study the association between the molecular structural features related to the amide region and protein utilization of blend-pelleted products based on canola meal or carinata meal, and (2) evaluate the effects of feeding newly developed blend-pelleted products based on carinata meal or canola meal on production efficiency, ruminal fermentation characteristics, ruminal degradability, and intestinal digestion in high-producing dairy cows. Result from the first study showed that the moleclar structural related to amide region were detected using fourier transform infrared (FTIR) vibration spectroscopy in which, increasing the level of canola or carinata meal in the blend-pelleted products (BPPs) significantly increased ($P < 0.05$) the amide area and amide height. All BPPs were similar in the secondary structure profile (α helix to β sheet ratio). A second study was conducted to investigate the effect of feeding BPPs based on canola and carinata meal relative to control diet (control, is a barley-based diet in western Canada) on production efficiency, nutrients digestibility, and nitrogen balance in dairy cows. The results showed that there was no significant effect ($P > 0.10$) of dietary treatments on milk yield, milk composition, and milk components yield. All dietary treatments exhibited the same income over feed cost ($P > 0.10$). The total-tract digestibility of nutrients and nitrogen balance were not ($P > 0.10$) affected by treatments. Third study was carried out to assess the effect of the dietary treatments on ruminal fermentation and ruminal digestion in dairy cows. The control diet exhibited a higher rumen total volatile fatty acid concentration ($P < 0.05$) relative to BPP based on canola meal. There was no effect ($P > 0.10$) of treatments on ruminal ammonia concentration. Furthermore, all diets exhibited the same ($P > 0.10$) ruminal degradation kinetics, intestinal digestion of nutrients, and metabolizable protein supply in dairy cows. In conclusion, the blend-pelleted products based on new co-product (carinata meal) from bio-fuel processing industry

is similar to the other pelleted products based on canola meal without affecting the production efficiency or the ruminal fermentation features in dairy cows. Molecular spectroscopy can be used to determine the inherent structural characteristics in relation to protein profile, energy values, protein digestion (rumen and intestine), and the metabolizable protein supply in the blend-pelleted products based on different bio-energy co-products.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BDOM	Rumen bypass organic matter
BDST	Rumen bypass starch
BPP	Blend-pelleted products
CA4	Sugar (rapidly degradable carbohydrate fraction)
CB1	Starch (intermediately degradable carbohydrate fraction)
CB2	Soluble fiber (intermediately degradable carbohydrate fraction)
CB3	Digestible fiber (available neutral detergent fiber, slowly degradable carbohydrate fraction)
CC	Indigestible fiber (unavailable neutral detergent fiber)
CF	Crude fiber
CHO	Carbohydrate
CP	Crude protein
CNCPS	Cornell Net Carbohydrate and Protein System
D	Degradable fraction
dBDM	Intestinal digestibility of rumen bypass dry matter
dBOM	Intestinal digestibility of rumen bypass organic matter
dBST	Intestinal digestibility of rumen bypass starch
DE_{p3x}	Digestible energy at level (3x maintenance)
DM	Dry matter
DPB	Degraded protein balance
DVE	Total truly digested protein in the small intestine (DVE/OEB system)
ECM	Energy-corrected milk
EDCP	Effective degradability crude protein
EDDM	Effective degradability dry matter
EDOM	Effective degradability organic matter

EDST	Effective degradability starch
EE	Ether extract
FCM	Fat-corrected milk
FMV	Feed milk value
FTIR	Fourier transform infrared spectroscopy
IDBDM	Intestinal digestible rumen bypass dry matter
IDST	Intestinal digestible rumen bypass starch
IDP	Intestinal digestibility of protein
iNDF	Indigestible neutral detergent fiber
Kd	Degradation rate of potentially degradable fraction
Kp	Passage rate
MCP	Microbial crude protein
ME	Metabolizable energy
ME_{p3x}	Metabolizable energy at a production level (3x maintenance)
MP	Metabolizable protein
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE	Net energy
NEL_{P3x}	Net energy at a production level (3x maintenance)
NFC	Non-fiber carbohydrate
NRC	National Research Council
OM	Organic matter
PA2	Soluble true protein (rapidly degradable protein)
PB1	Insoluble true protein (moderately degradable protein)
PB2	Fiber-bound protein (slowly degradable protein)
PC	Indigestible protein
PCA	Principal components analysis
RDP	Rumen degradable protein
RUDM	Rumen bypass or undegraded feed dry matter
RUOM	Rumen undegradable of organic matter
RUP	Rumen undegradable protein
SCP	Soluble crude protein
T_0	Lag time
tdCP	Total truly digestible crude protein

tdNDF	Total truly digestible neutral detergent fiber
TDN	Total digestible nutrients
TDN_{1x}	Total digestible nutrients at a maintenance level
TMR	Total mixed ration
TRDC	Total degraded ruminal carbohydrates fraction
VFA	Volatile fatty acid

1. GENERAL INTRODUCTION

Bio-energy processing such as bio-fuel, bio-oil, and bio-ethanol industries resulted in huge amounts of co-products such as canola meal, carinata meal, and distiller's dried grains with solubles (Ban and Yu, 2016; Canola Council of Canada, 2015; Xin and Yu, 2013a). Canola meal from bio-oil processing is the most common feed protein in western Canada and is characterized by moderate protein content (about 36-39% crude protein (CP); Canola Council of Canada, 2015). The new co-product from bio-fuel processing, i.e., carinata meal has been found to contain a higher protein content relative to canola meal (about 48% CP; Xin and Yu, 2013a) and good amino acids profile (Guevara et al., 2018). Pea is a good source of protein (approximately 24% CP) and contains a high level of starch (about 46% DM; Hickling et al., 2003). However, to maximize the utilization of these products and co-products in dairy cows, the rate and extent of protein digestion should be decreased to improve their N metabolism in cows.

The most common methods for maximizing the utilization of N are heat and chemical treatments. Heat treatments such as pelleting have been reported to improve the nutritional and chemical characteristics of protein feed (Lević et al., 2010). Chemical treatments, such as formaldehyde (Crooker et al., 1983), tannins (Chung et al., 2013), lignosulfonate, and xylose (McAllister et al., 1993) can decrease rumen degradable protein for different protein sources in dairy cows.

Traditional methods of analysis i.e. “Wet” chemistry analysis for protein and carbohydrates fractions can give us the information about the nutrient composition of a feed, but it can damage the main structure of samples (Yu et al., 2014). Fourier-transform infrared spectroscopy (FTIR) is commonly used to determine the molecular structure features of feeds. FTIR spectroscopy is a direct, rapid, non–destructive, and non–invasive bioanalytical technique used to detect the infrared

spectrum of absorptions or emissions of liquid, gas, or solids (Smith, 2009). However, there is no systematic study that has been conducted to determine how the internal protein molecular structures change when carinata meal is blended and pelleted with other co-products as a blend-pelleted product (BPP) and how these changes influence the protein utilization and availability in dairy cows. The main objectives of this thesis were to assess the effects of feeding blend-pelleted products based on new carinata meal or canola meal, pea screening, and lignosulfonate chemical compound on milk production efficiency, ruminal fermentation characteristics, ruminal degradability, and intestinal digestion in high producing dairy cows. Also, to detect the interactive association between the molecular spectral features related to amide region and production performance and ruminal fermentation characteristics of BPPs in high producing dairy cows.

2. LITERATURE REVIEW

2.1. Development of Canola Co-Product

2.1.1. Features of Canola Meal from Bio-Oil Processing

Canola is Canada's main crop, currently ranked amongst the top three oilseeds worldwide (Thiyam-Hollaender et al., 2013). Canada has the highest production of canola oil worldwide; Canada produces about 15 million tonnes of canola seeds per year (Canola Council of Canada, 2015). Saskatchewan was the first Canadian province to produce canola oil (Thiyam-Hollaender et al., 2013).

Canola or double-zero rapeseed is an offspring of rapeseed, bred through traditional plant breeding between *Brassica napus*, *Brassica rape or Brassica juncea* (Bell 1993; Newkirk, 2009) such that the oil contain low concentration of erucic acid (<2% erucic acid in its fatty acid profile) and low conntration of glucosinolates (< 30 µmol) in the solid component (Bell 1993). The most

common species of canola in western Canada are *Brassica juncea* and *rapa* (yellow-seeded) and *Brassica napus* (brown-seeded; Newkirk, 2009).

2.1.2. Utilization of Canola Meal in Ruminant Livestock

Canola meal is divided into the yellow-seeded and the brown-seeded species. The protein content in the yellow-seeded species is higher than in the brown-seeded species (Theodoridou and Yu, 2013b). Canola meal is an excellent palatable protein source for ruminant animals. Canola meal contains about 36-39% CP (N×6.25, %; Canola Council of Canada, 2015).

The nutrient composition of canola meal includes: dry matter (DM) 88 %; CP 36.7 %DM; neutral dteregent fiber (NDF) 25.4 %DM; acid detergent fiber (ADF) 16.2 %DM; lignin (ADL) 5.8 %DM; ether extract (EE) 3.3 %DM; starch 5.1 %DM; ash 6.7 %DM. Canola meal is a great source of the amino acids (AAs) such as lysine (5.92 %CP), histidine (3.39 %CP), methionine(1.95 %CP), cystine (2.31 %CP), and threonine (4.27 %CP; Canola Council of Canada, 2015).

It is essential to provide the dairy cows with an adequate level of rumen degradable protein (RDP) and rumen bypass protein (RUP) in the diet (NRC, 2001). The RDP level is vital to maximize the microbial protein synthesis (NRC, 2001). It has been reported that any decrease in the RDP content of the ration below the recommended 10% of DM (NRC, 2001) could reduce microbial protein synthesis due to a lower ruminal NH_3-N and total free amino acids (Brito et al., 2007; Broderick et al., 2007). The RDP of canola meal had been reported to range from 55% to 60%CP (Mustafa et al., 1996; Piepenbrink and Schingoethe, 1998).

There is no limit regarding the inclusion level for canola meal in dairy cow ration (Canola Council of Canada, 2015). For instance, it has been found that milk production of dairy cows was maintained for over 44 kg/cow/d, with diets containing 20% canola meal (Swanepoel et al., 2014). Brito et al. (2007) reported that replacing 12% soybean meal and 4.5% corn meal with 16.5%

canola meal in diets for high-producing cows increased the dry matter intake. Brito and Broderick (2007) also showed adding 16.5% of canola meal inplace of soybean (12%) and corn meal (4.5%) into diets led to increasing the milk yield of dairy cows.

2.1.3. Conventional Canola Meal Processing

The co-product from the bio-oil processing of canola seed is canola meal. Processing of canola seeds is called pre-press solvent extraction (Canola Council of Canada, 2015; Newkirk, 2009), which includes the following steps: (1) cleaning the seeds from the dockage materials and crush those seeds; (2) drying the seeds at approximately 35 to 45ºC for 35 to 45 min before flaking; (3) flaking the seeds by roller mills to rupture the seed coat without damaging the quality of the oil; (4) cooking the flakes at 80-105ºC for 15-20 min; (5) pressing the cooked seeds flakes to remove as much oil as possible from the cooked canola (removing about 50-60% of the seed oil content) to produce the presscake. Because the pressing process cannot remove all oil from the seed (the remaining oil 18-20%), solvent extraction is performed to remove oil from the remaining canola presscake; (6) solvent extraction includes the following steps: first, the cake is placed in the extractor, then the cake is flooded with solvent or miscella, then a sequence of pumps sprays the miscella over the presscake with each stage using a successively "leaner" miscella to increase the ratio of solvent to oil. Afterward, the solvent infiltrates by gravity through the cake bed, diffusing into and soaking the cake fragments. Finally, the marc (hexane-saturated meal) that leaves the solvent extractor, after a fresh solvent wash, contains low content of fat (2-4%; Newkirk, 2009).

2.2. Development of Carinata Co-Product

2.2.1. Features of Carinata Meal from Bio-Fuel Processing

Brassica carinata is a species of Brassica family, created from hybridization between *Brassica nigra* and *Brassica oleracea* (Warwick et al., 2006). It is commonly called Ethiopian

mustard (Rakow, 2004). Agriculture and Agri-Food Canada (AAFC) have successfully grown *Brassica carinata* since the mid-1989s in the dry prairie of western Canada: Alberta, Saskatchewan, and Manitoba (Rakow and Getinet, 1998; Taylor et al., 2010; Ban and Yu, 2016). This crop has been found to produce a high yield with high oil content in these areas, regardless of heat and drought; also, this crop shows good salinity tolerance and blackleg resistance (Rakow and Getinet, 1998; Taylor et al., 2010; Ban and Yu, 2016). Canada has two developed species of carinata bred by AAFC. The AAC A100 seed was released in 2012, and small quantities of AAC A110 seeds were available in 2015 (Resonance Carinata, 2015).

The AAFC carinata seed comprises approximately 44% oil and 28% CP (Resonance Carinata, 2012), and it has a high level of erucic acid (>30% of total fatty acids; Warwick et al., 2006). The high level of erucic acid in carinata seeds is utilized in bio-fuel processing industry (Cardone et al., 2003). The yellow seeds of carinata have a higher protein content than the brown seeds of carinata (Simbaya et al., 1995). Carinata meal is a source of crude protein, which could reach about 48 %CP (Xin and Yu, 2013b).

Carinata seed has higher anti-nutritional compounds such as glucosinolates (119.8 umol/g) and erucic acid (42.1%). Both of these compounds can negatively affect cattle health (Getinet et al. 1996; Warwick et al. 2006). However, the type of seed processing which uses high temperature as in pre-press solvent extraction has resulted in a meal with erucic acid (Newkirk et al. 2003b).

2.2.2. Conventional Carinate Meal Processing

The co-product from bio-fuel processing of *Brassica* carinata seed is carinata meal (Edwards et al., 2011). During the bio-fuel processing of carinata seeds, the oil in the seeds is extracted by conventional crush infrastructure with minimal refining once crushed and filtered the seeds (Edwards et al., 2011).

2.2.3. Utilization of Carinata Meal in Ruminant Livestock

The nutrient composition of carinata meal includes: DM 88.5 %; CP 44.3 %DM; NDF 23.7 %DM; ADF 16.3 %DM; ADL 5.9 %DM; EE 2.1 %DM; starch 2.3 %DM; non-fibrous carbohydrate 24.5 %DM; ash 7.6 %DM; glucosinolates 11.5 μmol/g (Ban, 2016). Although there is a high level of glucosinolates in carinata seeds, increasing heat or time under heating during the bio-fuel processing of carinata seeds could decrease the glucosinolates content (Guevara-Oquendo, 2017). It has been found that the canola meal pellet has relatively higher levels of total glucosinolates than the carinata meal pellet (4.76 vs. 4.28 μmol/g; Guevara-Oquendo, 2017). Carinata meal is a rich source of amino acids, containing arginine (10.8 %CP), glutamic acid (20.7 %CP), and proline (6.5 %CP), but is lower in isoleucine (4.1 %CP), leucine (6.8 %CP), valine (4.9 %CP), tyrosine (2.5% CP), lysine (4%CP), and methionine (1.8 % CP) compared with canola meal (Ban, 2016).

The carinata meal has been reported to have a higher RDP level compared with canola meal (75 vs. 60 %CP; Ban, 2016). The rumen degradation rate of a potentially degradable fraction of CP is much higher in carinata meal (ranged from 33 to 22 h/%) than canola meal (ranged from 11 to 17 h/%; Ban, 2016; Xin and Yu, 2014).

2.3. Development and Production of Pulse Processing Co-Product

Pea (*Pisum sativum L.*) is a member of the Leguminosae family (Khorasani et al., 2001). Canada is the second country for producting peas (Hickling et al., 2003). Alberta has the highest production of peas in Canada. Canada uses peas for human consumption and animal feeding (Hickling et al., 2003). Saskatchewan has the greatest yield of the dry pea crop and chickpea crop in Canada (Saskatchewan Pulse Growers, 2015).

2.3.1. Pulse Peas Processing and Their Co-Products

Co-products from the pulse processing industry include pea screenings that after cleaning of foreign materials (Yu et al., 2002). The material obtained is dockage (the material removed after cleaning includes chaff, other grain, weed, or inseparable seeds, and pieces of a stem). After cleaning dockage , the pulse peas processing will produce three products, including No. 1, No. 2, and No. 3. Refuse screenings of peas are No. 3 product. No. 1 and No. 2 products are relatively high in value (McKinnon, 2015). Peas are a source of protein and energy (a high starch level).

2.3.2. Utilization of Pulse Peas and Co-Products in Ruminant Livestock

The CP in field peas is approximately 24% (Fonnesbeck et al., 1984). The nutrient composition of peas includes DM 90 %; CP 23 %DM; CF 5.5 %DM; starch 46 %DM; ash 3.3 %DM (Hickling et al., 2003). It contains a high level of starch about 47.8 % (Valentine and Bartsh, 1987). Peas have high levels of essential amino acids, such as histidine (2.52 %CP), methionine (1.03 %CP), cystine (1.55 %CP), and threonine (3.59 %CP); also, by comparing the pea protein with the cereal grains and most oilseed meals, pea protein is the highest in lysine (6.84 %CP). Also, peas have an appropriate amino acid balance (Hickling et al., 2003).

The RDP of peas is high, roughly estimated to be about 78% RDP as a % of CP. Thus it could meet the microbial N requirements of lactating dairy cows (Kudlinskiene et al., 2016). The remaining moderate amount of RUP with a good AA (Lysine and Methionine) balance is good for milk production in high-producing dairy cows (Kudlinskiene et al., 2016). Previous study showed that replace soybeans with peas in dairy cows diets resulted in an increasing of milk fat and protein content, but it had a negative effect on milk yield (Kudlinskiene et al., 2016).

2.4. Strategies to Improve the Utilization of Co-Products

It important to slow down the degradation (extent and rate) of ruminal degradation of feed proteins for many reasons (Schwab, 1995). For example, there are many feeding situations where

the ration does not provide acceptable absorbed AA supply compared to the absorbed energy supply (Schwab, 1995). This could take place due to the following reason; first, several feed components in the ration contain an inadequate amount of RUP compared to RDP; second, dietary shortage in fermentable carbohydrates or RDP that are required for microbial protein synthesis; third, providing dairy cows with fat supplement rich in metabolizable energy but not for microbial cell growth (Schwab, 1995). Also, if feeding high-quality forages that contains a high level of RDP, it is crucial to provide the diet with an adequate amount of RUP to balance RDP and RUP (Schwab, 1995). The utilization of rich undegradable protein sources would enhance the efficiency of non protein nitrogen (NPN) supplements and would have less dependence on more degradable protein sources of true protein for microbial protein synthesis in the rumen (Schwab, 1995).

There are many approaches for raising the proportion of RUP in the diet. One method is to provide dairy cows with high-protein co-product feeds, such as corn gluten, meat, hydrolyzed feather, fish, and blood meals (Chalupa, 1975; Waldo, 1977; Kaufmann and Liipping, 1982; Broderick et al., 1991). The issues in using these co-products are their higher cost and lower commercial availability, uniformity, AA balance, and intestinal digestibility and palatability of product dictate their use. Another approach is artificially decreasing the rate of ruminal degradation of high quality protein sources with a good AA profile and better intestinal availability, which are rapidly degraded (Chalupa, 1975; Waldo, 1977; Beever and Thomson, 1981; Kaufmann and Liipping, 1982; Broderick et al., 1991). This approach would have the advantage protecting the AA from ruminal degradation and maximize their utilization in dairy cows (Chalupa, 1975; Waldo, 1977; Beever and Thomson, 1981; Kaufmann and Liipping, 1982; Broderick et al., 1991). The following section highlights the most common methods successfully used to maximize protein utilization and protect the AA.

2.4.1. Heat-Related Treatments to Improve Nutrient Utilization

It is essential to use heat treatments to improve the nutritional, hygienic, chemical, physical, and other animal feed characteristics (Lević et al., 2010). Heat treatment can modify the amino acid residues of proteins by reacting with other compounds or through cross-linking, and this reaction decreases ruminal protein degradation, protecting the proteins from hydrolytic activities of rumen microbiota (Petit et al., 1999). Therefore, heating tends to provide a more gradual release of protein within the rumen, enhancing the digestibility of nutrients and the milk production of dairy cows (Petit et al., 1999).

There are different types of heat treatment, and each type is unique in terms of heat source, the structure of the system, and its efficiency. The temperature and the heating time are the two main mutual factors among all heat treatments (Yu et al., 1998). Most procedures that are used are hydrothermal treatments. The main types of heat treatments in animal feed processing include dry roasting, steam flaking, pelleting, extrusion, etc. (Jansen, 1991; Riaz, 2007).

2.4.1.1. Dry Roasting

Roasting is dense, dry heating of raw material under temperature 110 - 170°C (Kumar et al., 2015). The temperatures used depend on the device used and the desired product quality. If the temperature of roasting is too high, it could lead to reducing the availability of nutrients in the surface layers of grain (Kumar et al., 2015). One of the main objectives of dry roasting is to improve energy availability. It deactivates enzymes and inhibiting factors, enhancing the feeding value of the feedstuff (Kumar et al., 2015).

The effect of roasting on feed utilization is more pronounced in ruminant studies compared to non-ruminants, where it has been reported that the roasting had decreased the RDP of barley, corn, oats, and wheat in ruminant's diets (McNiven et al., 1994). The roasting of the soybean meal

increased the RUP and decreased protein degradation (rate and extent) in the rumen (McNiven et al., 1994). The reduction of protein degradation in the rumen is attributed to the Maillard reaction between free amino groups and sugar aldehydes (Dhiman et al., 1997). The roasting process has also been found to increase starch gelatinization (in corn) and decrease nitrogen solubilization in ruminants, resulting in improved microbial synthesis, increasing body weight gain and feed efficiency of utilization in calves (Sinclair et al., 1993; Abdelgadir et al., 1996). Dry roasting of faba beans was effective in shifting the CP degradation from the rumen to the intestine, hence, decreasing nitrogen losses in the rumen (Yu et al., 1998).

Numerous protein sources subjected to roasting processing have been found to reduce or to deactivate anti-nutritional factors. Roasting processing can modify the structure of the protein (denaturation), causing the deactivation of protein sources' anti-nutritional factors (i.e., trypsin inhibitors, lectins, etc.), because these proteins require their structural integrity to employ the effects (Van der Poel et al., 1990).

Although roasting is a typically an inexpensive method of heating, uneven heating has often resulted in inconsistent results. Some studies (Scott et al., 1991) have detected no effect on increased milk production when cows were fed roasted soybeans (meal or raw soybeans). But other researchers (Faldet et al., 1991) observed increases in milk production with roasted soybeans (meal or raw soybeans).

2.4.1.2. Pelleting

Pelleting is a feed processing technology, where the smaller particles of feedstuff are agglomerated into larger particles by using moisture, heat, and pressure (Falk, 1985). The pelleting process includes the following steps, first, crashing the larger feed portion into smaller feed portion and passing the mixed ground mash through the conditioner. The mash is exposed to some pre-

treatments before granulation, such as mixing with molasses or fats, conditioning with steam to enhance the binding ability, softening the feed, denaturing protein, and gelatinizing starch. Then, the feed is passed to the pelleting chamber and pressed through a die to make pellets. The temperature of pellets after leaving the die is generally higher (60 to 95ºC). Finally, pellets are cooled with ambient air (Thomas and van der Poel, 1996).

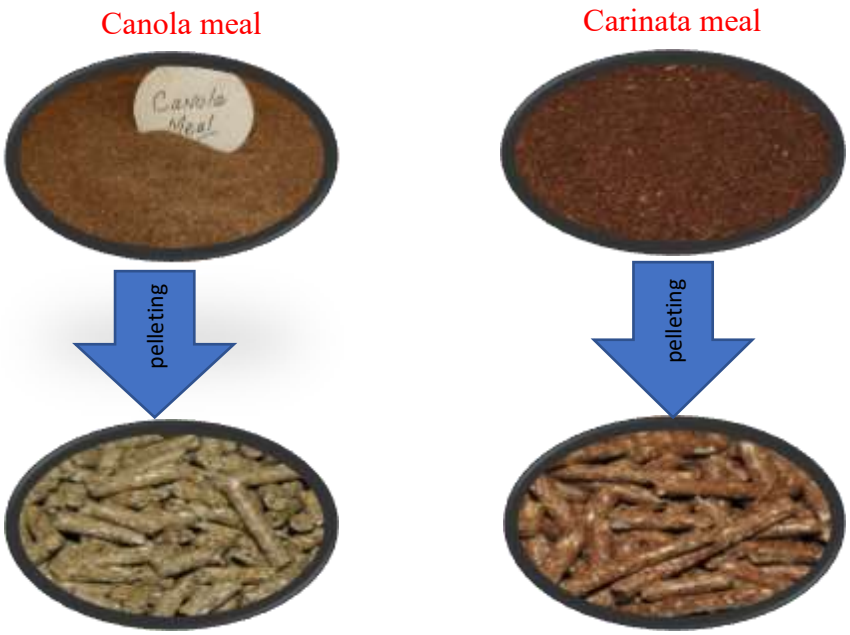

Figure 2.1. An example of blend pelleted products based on canola meal and carinta meal that has been developed to improve the protein utilization in dairy cows (Guevara-Oquendo, 2017).

The excellent physical quality of a pellet is characterized by the ability of the pellet to tolerate the fragmentation and abrasion during the mechanical and the pneumatic handling without breaking up the feed or without generating a high proportion of fines (Cramer et al., 2003). The pellet durability index (PDI) and pellet hardness are the two most parameters in use to estimate the physical quality of pellets (Thomas and van der Poel, 1996). The PDI can be measured by the

"Pfost" procedure (Thomas and van der Poel, 1996) or by using the Holmen pellet durability tester. In the Holmen pellet durability tester, the air is used to create abrasion of the pellets opposite the tumbling action (ANAC, 2013). Pellet hardness is another measurement of pellet quality and can be defined as the necessary force to crush a pellet. The "Kahl" device is used to measure pellet hardness (Abdollahi et al., 2013).

Using pellets in the feed industry and animal nutrition has many benefits, by increasing the bulk density and transfer efficiency of feed more than mash feeds (Thomas and van der Poel, 1996). The pelleting process could reduce microbial bioactivity, improve the health status of animal feed (Abdollahi et al., 2013), increase feed palatability (Abdollahi et al., 2013), inhabit the adverse effect of anti-nutritional factors (i.e. glucosinolates) by making them inactive (Abdollahi et al., 2013), improve rumen crude protein degradation in dairy cows (Goelema et al., 1999), and increase resistance of starch degradation in the rumen (Tamminga and Goelema, 1995; Huang, 2015).

2.4.1.3. Extrusion

The processing of extrusion includes pushing the feedstuff through the barrel by using means of screws of several formations and then pressing them through the die at the end of the barrel (Lević et al., 2010). The requisite of extrusion processing exposes the feedstuff to high temperature in a short time, where extrusion processing includes heating the feed in 155°C for 43 seconds by using a Multi-purpose twin-screw extrusion system (Lević et al., 2010). Due to the different pressure between the inside of the extruder and the external environment, it will lead to a partial evaporation of water at the exit point and the development of the product (Lević et al., 2010). However, extrusion processing is a complex technology, but it is very flexible in the same

time, where it provides the processing of a variety of several raw materials such as soybean, sunflower, rapeseed, wheat, corn, barley, oats, beans, peas, etc. (Smoje et al., 1996).

Extrusion could protect the dietary protein from microbial degradation in the rumen. For example, extrusion of oilseeds, such as canola seed, leads to increases in the production of milk in dairy cows (Ingalls and Grumpelt, 1987). In addition, extrusion of lupin seeds could reduce the degradability of crude protein in the rumen, enhancing the nutritive value of seed, such as the source of undegraded protein (Cros et al., 1992). Studies on extrusion process reported ineffective in improving the post-ruminal supply of amino acids from flaxseed-based diets (Mustafa et al., 2003) because of increasing the ruminal CP digestibility and reducing the quantity of CP supply for post-ruminal digestion for cows fed diets of extruded flaxseeds (Mustafa et al., 2003). Nevertheless, the impact of extrusion processing on CP digestion in the rumen could vary if the processing procedure is modified, particularly the temperature used for the processing procedure and resident time during the processing, which may alter the effect of extrusion on CP digestion for different flaxseed (Mustafa et al., 2003).

2.4.2. Chemical Treatments to Improve Nutrient Utilization

Many chemical factors can lead to decreasing the RDP content of protein in different feeds. Many studies have been attempted to increase the proportion of RUP reaching the small intestine of ruminants by treatments with formaldehyde (Crooker et al., 1983), tannins (Chung et al., 2013), lignosulfonate (LSO_3), and xylose (McAllister et al., 1993).

2.4.2.1. Lignosulfonate

Lignosulfonate is a feed additive that can be used as a pellet binder in animal feed to improve pellet quality (Corey et al., 2014). Lignosulfonate (Calcium lignosulfonate) has been used industrially in several applications. Windschitl and Stern (1988) reported that adding

lignosulfonate (LSO_3) to soybean meal, followed by heating at 90-95 ºC for 45 min, decreased CP digestion in the rumen. Canola meal treated with 7% LSO_3 heated to 100°C increased rumen escape protein content (McAllister et al., 1993; Stanford et al., 1995). In addition, the treatment of canola meal with 5% LSO_3 heated to 100°C for 60 min and 25% moisture (moist heat) resulted in a higher reduction in effective rumen degradability of CP than heat treatment without LSO_3 (McAllister et al., 1993). Other studies (Mansfield and Stern, 1994; Stanford et al., 1995) reported that the organic matter digestibility of soybean meal or canola meal was not affected by LSO_3 supplementation. Güçlü (1999) reported that LSO_3 supplementation decreased DM digestibility of cottonseed meal. Supplementation of LSO_3 would have a beneficial effect in inhibiting the adverse impact of anti-nutritional factors in the feed. Guevara-Oquendo (2017) has found that adding lignosulfonate to blend-pelleted products of canola meal, or carinata meal could reduce the total level of glucosinolates in blend-pelleted products.

2.4.2.2. Tannins

Tannins are primarily considered anti-nutritional biochemicals due to their adverse effects on feed intake and nutrient utilization (Kumar, 1990). In recent years, tannins have been recognized as beneficial phytochemicals for modulating rumen microbial fermentation (Kumar, 1990). Many researches have reviewed the effects of tannins on ruminants, focusing mostly on adverse effects of tannins on animal systems with some discussion of the beneficial effects of tannins addition on prevention of bloat and protein degradation (Mueller-Harvey, 2006; Waghorn, 2008). In review by Mueller-Harvey (2006) and Waghorn (2008), little mention of methane inhibition by tannin-containing forages. More recently, a high number of studies have been published on the effects of tannins on inhibiting the methanogenesis and decreasing the protein

degradability in the rumen, justifying fresh appraisal of the present scenario on the influences of tannins on rumen metabolism and animal performance.

The tannins are divided into condensed tannins and hydrolyzed tannins (Khanbabaee and Ree, 2001). Though, as a rule, hydrolyzable tannins have ability to bond with feed protein, may be degraded in the rumen enzymatic hydrolysis into several structural units (mainly phenolic acids), and have a lower capacity of attachment to feed protein (Khanbabaee and Ree, 2001). Instead, condensed tannins have higher stability in the rumen compared to hydrolyzed tannins, where they have a higher resistance to ruminal enzymes. The stability of condensed tannins are attributed to their high molecular weight; meanwhile, this could decrease their capacity to bond with feed proteins when compared to hydrolyzable tannins (Frutos et al., 2004). Frutos et al. (2004) found the condensed tannins decreased the degradability of soybean meal significantly when compared to commercial tannic acid. Hervas et al. (2000) treated soybean meal with different doses of tannic acid condensed tannins extract and found both treatment doses reduced the extent of crude protein degradation in the rumen.

2.5. Molecular Analysis of Feed Using Vibrational Fourier Transform Infrared (FTIR) Spectroscopy

2.5.1. The Basic Principle of Fourier Transform Infrared (FTIR) Vibration Spectroscopy

FTIR spectroscopy is based on the mathematical Fourier-transformation method and interferometry (Stuart, 2004). Every FTIR machine is based on an interferometer (Smith, 2011). The most common interferometer is the Michelson interferometers, which consists of four arms. The top arm is composed of a collimating mirror and an infrared source. The bottom arm includes a fixed mirror. The left arm contains a moving mirror. The right arm is where the samples and the

detector are located, while a beam splitter is placed in the middle of the interferometer (Smith, 2011). The IR beam from the infrared source emits to the collimating mirror and then produces parallel rays across the beam splitter (Smith, 2011). After that, the beam is split by the beam splitter to two beams (one beam (50%) moves to the fixed mirror, and the other one (50%) moves towards the moving mirror; Smith, 2011). The reflected beams by the steady and moving mirrors will encounter at the beam splitter and recombine to form a final beam. The combined beam will pass through samples after leaving the interferometer and receiving a combined beam by the detector to produce an interferogram (Smith, 2011).

The Fourier transformation method processes the interferogram and analyzes the signal frequencies (Stuart, 2005; Smith, 2011). By comparing the FTIR with the other types of infrared instruments, the FTIR can provide fast, easy, and exact gauges with good signal-to-noise ratios (SNRs). The FTIR needs little sample preparation, saving labor. The FTIR lets high throughout and multiplex scans (Stuart, 2005; Smith, 2011).

Recently, in feed science, it has become important to utilize the FTIR to reveal structural changes of molecules and confirmation of biopolymers among several types of feed stuff in relation to the nutrient values and nutrient utilization (Theodoridou and Yu, 2013; Xin and Yu, 2013c). For instance, the FTIR could reveal the variances between the components of feed, feed-based crop varieties, the impact of gene modification and processing of feed on spectral characteristics, and impact of protein and CHO degradation in rumen related structure (Theodoridou and Yu, 2013; Xin and Yu, 2013c).

2.5.2. Application of FTIR Spectroscopy in The Feed Analysis

The feeding value and fermentation features of animal feedstuff have been reported to be influenced by the inherent molecular structure (Yu, 2012b). The infrared spectroscopy can detect

and identify molecular information of feed (Yu, 2012b). Molecular spectroscopic methods, such as FTIR, is a rapid, direct, non-destructive, and non-invasive bioanalytical technique used to detect the infrared spectrum of absorptions or emissions of liquid, gas, or solids (Smith, 2011). The FTIR comprises three essentials spectrometer elements: (1) the radiation source; (2) the interferometer; (3) the detector (Hsu, 1997).

In recent years, studies in animal feed science reported the success of FTIR to reveal structural changes of molecules for different types of feed in relation to nutrient values, nutrient utilization, and availability (Abeysekara et al., 2013; Peng et al., 2014; Xin and Yu, 2013a, b). For instance, the FTIR has been used to identify the molecular structure for different crop varieties, feed ingredients, and to study the impacts of feed processing on protein- and carbohydrate-related structures (Abeysekara et al., 2013; Peng et al., 2014; Xin and Yu, 2013a,b).

2.5.3. Univariate and Multivariate Analysis for Spectra

There are two common methods for spectral analysis, the univariate and the multivariate analyses (Yu, 2005, 2012b). The univariate analysis uses a mathematical parameter related to spectra, such as band height and area intensities, band frequencies, and the band intensity ratios (Yu, 2012b). The univariate analysis can be used to correlate with the chemical and biological features of feeds (Yu, 2012b). The drawback of the univariate analysis is its limited ability to analyze and compare massive spectral data. Multivariate analysis technique is favored (Yu, 2005). The multivariate analysis includes the hierarchical cluster analysis (CLA) and principal component analysis (PCA; Yu, 2005c).

The protein metabolism in dairy cows could be affected by the type of proteins and hydrolytic enzyme activities in the gastrointestinal tract and protein molecular structure (Yu and Nuez-Ortín, 2010; Huang et al., 2017). The protein secondary structures comprise α-helix and β-

sheet (Marinkovic and Chance, 2002). The primary molecular structure of protein (amide I and amide II and their ratio) and secondary structure of α-helix and β-sheet features may affect protein utilization, protein bioavailability, and digestive behavior in ruminants (Yu and Nuez-Ortín, 2010; Huang et al., 2017), mostly because molecular structure of protein influences accessibility of rumen bacteria and gastrointestinal tract enzymes, which affect protein values and protein availability (Yu and Nuez-Ortín, 2010).

2.6. Literature Review Summary, Overall Research Objectives, and Hypothesis

The co-products from bio-energy processing are often used as a source of protein supplement in the livestock industry. New co-products from bio-fuel processing of carinata seed have become available in Canada. However, to date, no study has been carried out to evaluate the effect of feeding this new feed in dairy cows relative to the conventional protein source such as canola meal or distillers' grains. Previous studies showed that the canola meal and carinata meal have a higher rate and extent of digestion in the rumen. Thus, it necessary to use appropriate methods for reducing the degradability in the rumen, i.e., using feed additive (lignosulfonate, tannins) and proper feed processing (heat treatment).

Using vibrational molecular spectroscopy with chemometrics, including univariate and multivariate techniques, would reveal information about the molecular structure features related to the N-utilization in dairy cows. However, to date, there has been no systemic study that has been carried out to evaluate an association among molecular structure feature related to the amide region in different blend-pelleted products based on bio-energy co-products and the nutrient bioavailability and production efficiency in dairy cows.

2.6.1. Project Hypotheses

- Blend-pelleted products that developed here can be used effectively as an alternative protein concentrates in replacing conventional diet that is based on soybean meal and peas ration in the dairy industry in western Canada without any adverse effect on milk production.
- Adding lignosulfonate (LSO_3) to the blend-pelleted products should improve protein digestion in the rumen and optimize nutrient supply to dairy cattle which could improve milk production of dairy cows.
- Processing-induced molecular structure changes in blend-pelleted products and blend-pelleted products based-total mixed ration will be significantly associated with nutrient utilization and availability and production performance of dairy cows.

2.6.2. Project Objectives

- To detect pelleting-induced molecular structural changes in terms of chemical functional groups related to the amide region in the blend-pelleted products based on a combination of carinata meal or canola meal with pea screenings, and lignosulfonate at different levels.
- To reveal the interactive association between the molecular spectral profiles related to amide region and the nutrient utilization and availability of blend-pellet products in dairy cattle.
- To determine chemical profiles, energy values, and nutrient fractions of protein, ruminal and intestinal utilization and availability of nutrients for the blend-pelleted products in high producing dairy cows.
- To examine the effect of feeding different blend-pelleted products on production performance, nutrients digestibility, nitrogen balance, ruminal fermentation characteristics, and ruminal pH profile in lactating dairy cows.

- To examine the interactive association between blend-pelleted products based-total mixed ration molecular spectral profile and nutrient digestion, nitrogen balance in high producing dairy cow.

3. INTERACTIVE ASSOCIATION OF MOLECULAR STRUCTURE OF BLEND-PELLETED PRODUCTS BASED ON COMBINATION OF NEW CO-PRODUCTS FROM BIO-ENERGY PROCESSING, PULSE SCREENINGS, AND LIGNOSULFONATE COMPOUND WITH PROTEIN UTILIZATION IN DAIRY COWS

3.1. Abstract

This study to determine the molecular structural features related to amide region and to quantify the relationship between molecular structural profile of amide region and protein bioavailability of blend-pelleted products (BPPs) based on canola meal and new bio-fuel co-products (carinata meal) with different proportions of pea screenings and lignosulfonate compound in dairy cows. The molecular structures of amide region were determined using the advanced vibrational molecular spectroscopy (FTIR). The results showed that increasing the level of canola and carinata meal in the blend products significantly increased ($P < 0.05$) the amide area and amide peak height of the BPPs. All BPPs exhibited similar protein secondary structures (α helix to β sheet ratio). Protein molecular structure profiles were highly-associated ($P < 0.05$) with the ruminal degradation and estimated intestinal digestion characteristics of the protein. In conclusion, the vibrational molecular spectroscopy could detect inherent structural characteristics in the BPPs based on different co-products from bio-energy processing. The molecular structural features related to the protein region were highly-associated with the protein utilization in dairy cows.

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3.2. Introduction

Due to the high worldwide demands of oils and fuel in industry, bio-energy processing (bio-fuel, bio-oil, and bio-ethanol) has resulted in large amounts of co-products such as canola meal, carinata meal, and distiller's dried grains with solubles (Ban and Yu, 2016; Canola Council of Canada, 2015; Xin and Yu, 2013a). Many studies have investigated the utilization of canola meal in ruminant or monogastric animals (Heendeniya et al., 2012; Huang et al., 2017; Theodoridou and Yu, 2013a). Nevertheless, to our knowledge, there is limited information that could be found in the literature on the carinata meal (a new co-product) when it is blended with other feedstuff to optimize its feeding value, particularly physicochemical or biopolymer functions.

There are several methods for feed evaluation, such as wet chemistry analysis; however, wet analytical techniques could damage the main structure of samples (Yu et al., 2014). The feeding value and fermentation features of feedstuff have been reported to be influenced by the inherent molecular structure (Yu, 2004). The IR spectral region (ca. 4000–800 cm^{-1}) has a strong characteristic vibrational transition compared with near-IR region, especially in the wavelength range between ca. 1800 and 800 cm^{-1}, which is called the "fingerprint region" (Liu, 2009; Yu, 2004). Vibrational spectroscopy such as FTIR is commonly used to detect the molecular structure of feed. The FTIR spectroscopy is a direct, rapid, non–destructive, and non–invasive bioanalytical technique used to reveal the infrared spectrum of absorptions or emissions of liquid, gas, or solids (Smith, 2009).

The FTIR spectroscopy has many advantages such as revealing the molecules structural changes of different types of feed and determining the nutrient utilization and bioavailability of feed in ruminants (Reynolds et al., 1994). Moreover, this technique could recognize the molecular

structure of different crop varieties, feed ingredients, and studying the effects of feed processing on protein and carbohydrate–related intrinsic structures (Abeysekara et al., 2013; Huang et al., 2015; Khan et al., 2015; Peng et al., 2014; Xin and Yu, 2013b). However, there is no systematic study that has been conducted to determine how blend-pelleted products (BPPs) based on different co-products of bio-oil or bio-fuel processing (i.e. carinata or canola meal), could induce changes in protein intrinsic molecular structures and how these changes influence the protein utilization in dairy cows. Therefore, the current study was performed to: 1) investigate the magnitude of differences among eight different BPPs from the bio-energy processing (carinata meal vs. canola meal) with different proportions of pea screenings and lignosulfonate in terms of protein molecular structure and 2) estimate the protein inherent structure changes in relation to protein profile, CNCPS protein sub-fractions, energy values, protein digestion (rumen and intestine), and the metabolizable protein supply in dairy cows.

3.3. Materials and Methods

3.3.1. Sample preparation

The experiment was performed at the Department of Animal and Poultry Science, University of Saskatchewan (Saskatoon, SK, Canada). The co-products of canola meal and carinata meal were used in BPPs in combination with different levels of pulse pea screenings and lignosulfonate compound. Eight blends were formulated; the BPPs from 1 to 4 are based on carinata meal (Agrisoma; Saskatoon, Canada) with different levels of pea screenings and lignosulfonate; and the BPPs from 5 to 8 are based on canola meal (Cargill Animal Nutrition, Clavet, Canada) with different levels of lignosulfonate and pea screenings. The composition of the BPPs (on dry matter (DM) basis) is as follow:

BPP1: lignosulfonate 0 % + carinata meal 50 % + pea screenings 50.0 % DM.

BPP2: lignosulfonate 4.8 % + carinata meal 47.6 % + pea screenings 47.6 % DM.

BPP3: lignosulfonate 0 % + carinata meal 75 % + pea screenings 25 % DM.

BPP4: lignosulfonate 4.8 % + carinata meal 71.4 % + pea screenings 23.8 % DM.

BPP5: lignosulfonate 0 % + canola meal 50 % + pea screenings 50.0 % DM.

BPP6: lignosulfonate 4.8 % + canola meal 47.6 % + pea screenings 47.6 % DM.

BPP7: lignosulfonate 0 % + canola meal 75 % + pea screenings 25 % DM.

BPP8: lignosulfonate 4.8 % + canola meal 71.4 % + pea screenings 23.8 % DM.

Pea screenings were sourced from ILTA Grain Company (Surrey, BC, Canada), while the lignosulfonate was obtained from Ameri-bond (Canada). The feed was produced in two different batches for each BPP. The pelleting was conducted at Canadian Feed and Research Centre (CFRC, North Battleford, Canada). For the pellet processing, the following procedure was followed to obtain the BPP: 1) Mixing the combinations in the Scott Equipment model TSM 363 (New Prague, MN, USA) for two minutes, 2) Heating the different combinations by using Colorado Mill Equipment ECO–R30 (Cañon City, USA) at 65°C and pelleting through a 3.6 mm diameter die such that the residence time of the blends in the die did not exceed 15 seconds, and 3) Cooling at room temperature.

3.3.2. Detection of blend-pelleted products impact on protein molecular structure changes

The detailed chemical composition, in situ rumen degradation profile, and the predicted nutrients supply to dairy cows, were previously reported by Guevara-Oquendo et al. (2018). The detailed chemical profile, Cornell Net Carbohydratevand Protein System (CNCPS) fraction, and energy values of the combined samples (n = 16) of BPPs (carinata meal or canola meal with different combinations of peas and lignosulfonate) are summarized in Table 8.1, 8.2 (Appendix).

These data were used for the correlation and regression studies. The crude protein (CP) was analyzed according to AOAC official method (984.13; AOAC, 1990). Neutral detergent insoluble crude protein (NDICP), non protein nitrogen (NPN), and acid detergent insoluble crude protein (ADICP) were estimated as described by Licitra et al. (1996). Soluble crude protein (SCP) was analyzed by incubating samples with bicarbonate–phosphate buffer then filtrating through Whatman filter paper (Roe et al., 1990). For energy profiles, total digestible nutrient (TDN), metabolizable energy (ME), digestible energy (DE), and net energy (NE) were used for estimating the available energy in BPP. The total digestible crude protein (tdCP), total digestible nutrients at maintenance level (TDN_{1x}), digestible energy at level 3x maintenance (DE_{3x}), metabolizable energy at level 3x maintenance (ME_{3x}), and net energy of lactation at level 3x maintenance (NEL_{3x}) were estimated by using a summative approach of the NRC (2001).

The in situ rumen degradation and the intestinal digestion of CP were performed in according to Yu et al. (2003). Degradation characteristics of CP were estimated by applying the first-order kinetics degradation model described by Orskov and Mcdonald (1979). The results were calculated using the NLIN procedure of SAS 9.4 with an iterative least-squares regression (Gausse Newton method):

$$R(t) = U + D \times e^{-Kd \times (t - T0)}$$

where R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (h−1) and T0 = lag time (h).

The rumen bypass crude protein (BCP) was estimated according to NRC (2001):

$$\% \text{ BCP} = U + D \times Kp / (Kp + Kd)$$

where, Kp stands for estimated passage rate from the rumen (h1) and was assumed to be 6 %/h for CP.

The predicted nutrient supply was estimated using the NRC (2001) model. In this model, the metabolizable protein (MP; g/kg DM) was calculated based on the following equation (NRC, 2001):

$$MP = AMCP + ARUP + AECP$$

where, AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine.

The feed milk value (FMV) was predicted based on the metabolizable protein content of BPP, where the efficiency of utilizing of metabolizable protein in dairy cows was assumed to be 0.67, and protein composition in milk was assumed to be 33 g protein / 1 kg of milk (NRC, 2001).

3.3.3. Fourier Transform Infrared (FTIR) Vibration Spectroscopy Analysis

3.3.3.1. Univariate Molecular Spectral Analysis of Protein Profile

For the molecular analysis, the samples were grounded to pass a 0.12 mm sieve (Retsch ZM200, Rose, Scientific Ltd., Canada) for FTIR spectroscopic analysis. Every sample was spectroscopically scanned for five times. The molecular spectral data of samples were collected and corrected for the background spectrum using FTIR molecular spectroscopy (JASCO 4200, JASCO International Co. Ltd., Tokyo, Japan). The spectra were generated in the mid–IR (ca. 4000–800 cm^{-1}) and the fingerprint region (ca. 1800–800 cm^{-1}) with a spectral resolution of 4 cm^{-1}. The FTIR spectra were processed by using OMNIC 7.3 (Spectra–Tech, Madison, WI). The regions of specific interest in this study included the primary molecular protein structural (amide I and amide II) and the secondary molecular protein structural (α–helix and β–sheet) in the mid-IR. The structural spectra information on the protein was determined by analyzing the absorption peak parameter such as region, baseline, peak, height, and area according to Yu (2004).

The univariate spectral analysis of protein structure comprised the primary and the secondary protein structures. The primary protein structures included amide I and II. The baseline of protein spectral was centered at ca. 1480–1730 cm^{-1} (Figure 3.1). The baseline of the amide I area was centered at ca. 1569–1730 cm^{-1} (Figure 3.2a). The baseline of the amide II area was centered at ca. 1480–1569 cm^{-1} (Figure 3.2c). The peak height of the amide I was centered at ca. 1638–1649 cm^{-1} (Figure 3.2b), while the peak height of amide II was centered at ca. 1533–1540 cm^{-1} (Figure 3.2d). The secondary protein structures of the amide I region were estimated by using the 2nd derivative funcitona and Fourier self–deconvolution function on OMNIC 7.4 Software (Spectra Tech, Madison, WI) according to previous studies (Theodoridou and Yu, 2013b; Yu, 2005). The secondary protein structures were comprised mainly α–helix and *β*–sheet. The peak height of α–helix was centered at ca. 1647–1653 cm^{-1}, while the peak height of *β*–sheet was centered at ca. 1625–1631 cm^{-1}.

3.3.3.2. Multivariate Molecular Spectral Analysis of Protein Profile

The principal component analysis (PCA) on FTIR data was performed using the Unscrambler 10.3 (CAMO Software AS, Oslo, Norway) for clustering any variation among blend-pelleted products. The raw data were preprossessed using baseline offset. The Savitzky–Golay algorithm was used to calculate the second derivative. Three principal components were selected for analysis. The two dimention (2D) plots were used to display the clustering among data sets. Loading points' plots for the most important principal components were used to display the relations among variables of data.

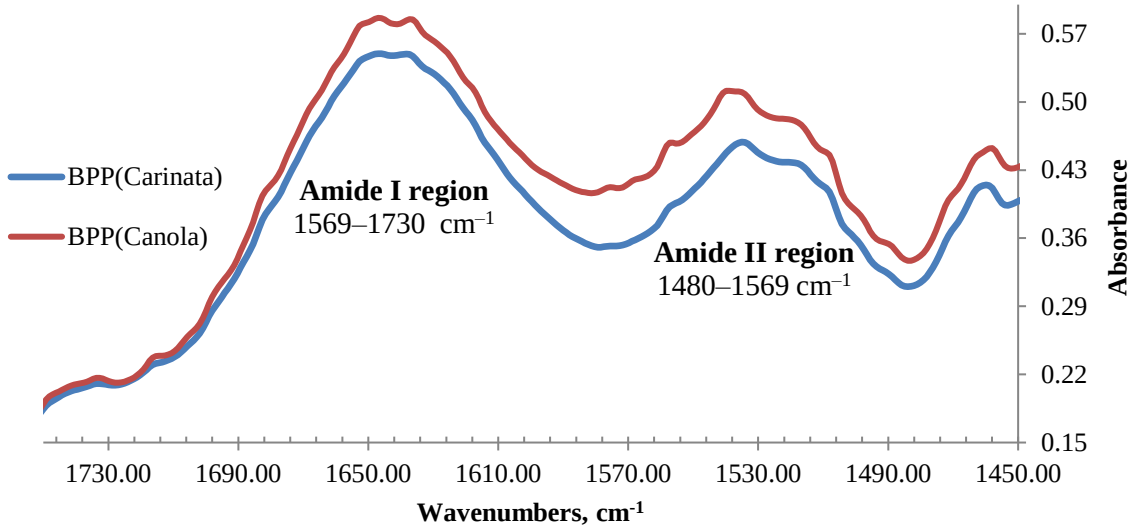

Figure 3.1. Typical Fourier transform infrared (FTIR) spectrum of the blend-pelleted products based on carinata or canola meal for protein region ca. 1730 to 1480 cm-1, showing the functional group makeup of protein amide I and II

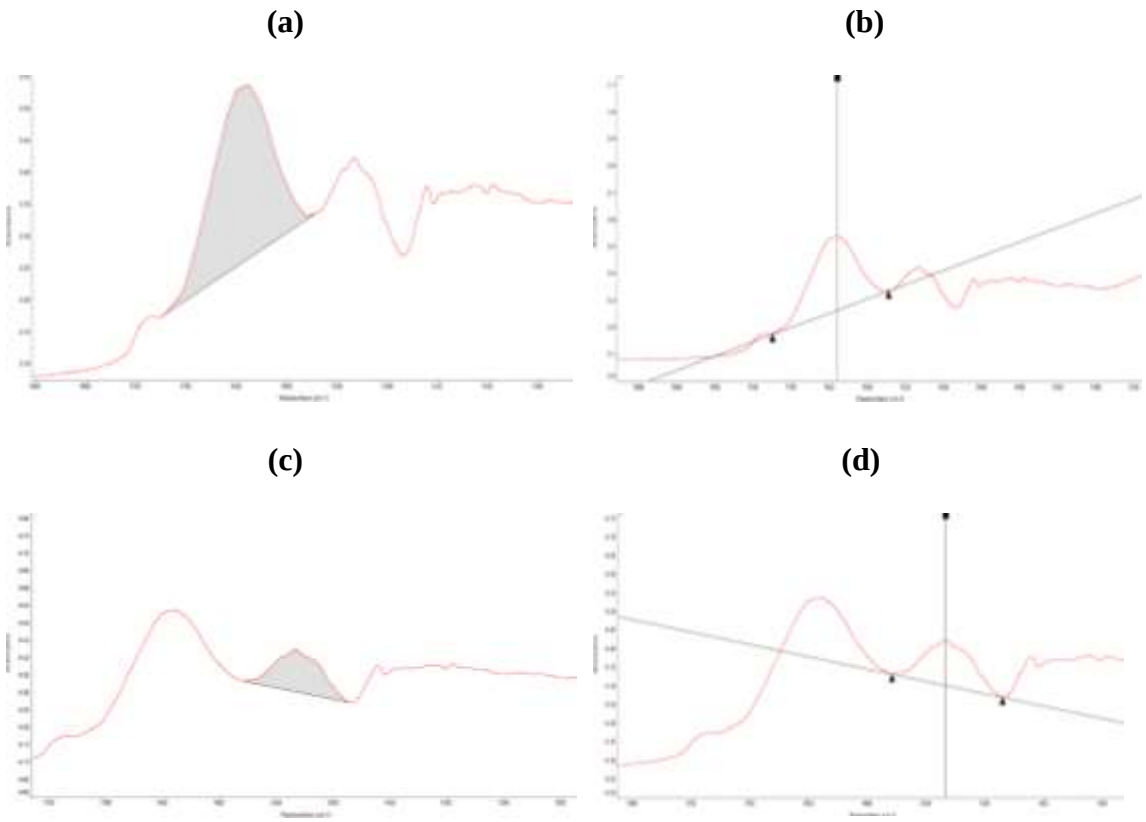

Figure 3.2. Spectral profile of the blend-pelleted products related to protein region (a) amide I area (ca. 1730-1569 cm^{-1}); (b) Amide I peak height (ca. 1638-1649 cm^{-1}); (c) Amide II area (ca. 1569-1480 cm^{-1}); (d) Amide II peak height (ca. 1533-1540 cm^{-1})

3.3.4. Statistical analysis

The data of chemical functional groups in the protein region (ca. 1480–1730 cm^{-1}) were analyzed by SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The experiment was designed using the randomized complete block design (RCBD) with pellet processing run as a random block effect. The RCBD model assumptions were checked by using a Residual Analysis. The residual normality was tested using Proc Univariate with Normal Plot option. Multi-treatment comparison was carried out by using Tukey method.

The correlation between the functional groups related to protein region (amide I, II peak hights and areas, α–helix, *β*–sheet and their ratio) and the chemical profiles of protein, energy values, rumen degradation kinetics parameters, intestinal digestive characteristics of protein, and the truly-absorbed protein supply was analyzed by using the PROC CORR procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Rank correlation with the SPEARMAN option and normality test with the UNIVARIATE option were used in the correlation study.

Multiple regression analysis (with model variable selection method) was performed to select the best functional groups that would explain the nutritive values of BPP using the PROC REG procedure of SAS with a reversed stepwise option. The following model was used for the multiple regression with model variable selection: model Y= spectral parameter 1 + spectral parameter 2 + spectral parameter 3 + spectral parameter 4 + ... + error. The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". All variables left in the final prediction models were significant at the 0.05 level. Residual analysis for multiple-regression was performed using the Residual Analysis and residual normality was checked using the Univariate procedure of SAS with Normal and Plot options.

3.4. Results

3.4.1. Protein spectral intensities of blend-pelleted products

The spectral protein profiles include the primary and secondary structures of protein of different BPPs based on canola meal or carinata meal with different level of lignosulfonate and pea screenings by using FTIR vibrational spectroscopy are shown in Table 3.1. The results showed That BPP7 and BPP3 had the highest ($P < 0.01$) amide I peak height (averaged 0.307 IU), while the BPP2 and BPP6 had the lowest values (averaged 0.259 IU). The amide I area was significantly different ($P < 0.01$) among BPPs, where the BPP7 and BPP3 had the highest amide I area (averaged 21.2 IU), whereas the BPP2 and BPP6 had the lowest values.

Our results showed that the ratio of amide I to II had a higher value ($P < 0.05$) in BPP based on carinata meal compared with canola meal (2.12 vs. 1.98). Furthermore, adding the pea screenings decreased ($P < 0.05$) the ratio of amide I to II in BPP based on carinata from 2.19 to 2.05, and in BPP based on canola from 1.95 to 2.02.

The secondary structures such as α–helix and *β*–sheet and their ratio of BPPs are presented in Table 3.1. It has been found that the α–helix, *β*–sheet height ratio was the same for all treatments ($P = 0.10$). This study showed that the ratio of α–helix to *β*–sheet decreased ($P < 0.05$) from 1.17 to 1.13 with decreasing the level of co-products in the BPPs.

3.4.2. Correlation analysis between the amide spectral features and nutrients profiles in the blend-pelleted products

The correlation analysis between the vibrational spectral features and protein profiles, protein sub-fractions, and the predicted energy values of BPPs are shown in Table 3.2. The CP had positive correlations with amide I area ($r = 0.70$, $P < 0.05$), total amide area ($r = 0.58$, $P = 0.02$),

Table 3.1. Molecular spectral features related to amide region for different blend-pelleted products (BPP)*.

CO-P	Pea	LSO_3	Treatment	Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix	*β*-sheet height	α: *β* ratio
CR	High	NO	BPP1	0.23^{cd}	0.13^{bc}	2.07^{bc}	19.02^{d}	6.46^{bc}	25.49^{de}	0.32^{cd}	0.27^{de}	1.17
CR	High	Add	BPP2	0.25^{e}	0.12^{d}	2.03^{bc}	17.50^{e}	5.76^{d}	23.26^{f}	0.29^{e}	0.25^{f}	1.17
CR	Low	NO	BPP3	0.30^{ab}	0.14^{b}	2.15^{ab}	21.25^{a}	6.53^{b}	27.78^{ab}	0.34^{b}	0.30^{bc}	1.14
CR	Low	Add	BPP4	0.28^{c}	0.13^{cd}	2.22^{a}	20.00^{c}	6.00^{cd}	26.01^{cd}	0.32^{cd}	0.28^{cde}	1.14
CN	High	NO	BPP5	0.28^{cd}	0.14^{b}	1.96^{cd}	19.03^{d}	6.40^{bc}	25.44^{de}	0.33^{bc}	0.29^{cd}	1.15
CN	High	Add	BPP6	0.27^{de}	0.14^{b}	1.93^{cd}	18.00^{e}	6.55^{b}	24.56^{e}	0.31^{d}	0.27^{e}	1.19
CN	Low	NO	BPP7	0.31^{a}	0.16^{a}	1.89^{d}	21.09^{ab}	7.64^{a}	28.73^{a}	0.37^{a}	0.33^{a}	1.12
CN	Low	Add	BPP8	0.29^{bc}	0.14^{b}	2.14^{ab}	20.22^{bc}	6.60^{b}	26.82^{bc}	0.35^{b}	0.31^{b}	1.11
SEM				0.006	0.004	0.070	0.335	0.213	0.429	0.007	0.007	0.020
P-Value				<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.18
Contrast P-value	CO-P		CR vs CN	0.04	<0.01	<0.01	0.52	<0.01	0.02	<0.01	<0.01	0.45
	LSO_3		No vs Add	<0.01	<0.01	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	0.67
	Pea		High vs Low	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 3.1. *Cont'd.* Molecular spectral profile related to amide region for different blend-pelleted products (BPP)[*]

[*]Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; SEM: Standard error of means; [a-f] Means with the different letters in the same column are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; BPP1: lignosulfonate 0 % DM + carinata meal 50 % DM + pea screenings 50.0 % DM.; BPP2: lignosulfonate 4.8 % DM + carinata meal 47.6 % DM + pea screenings 47.6 % DM; BPP3: lignosulfonate 0 % DM + carinata meal 75 % DM + pea screenings 25 % DM; BPP4: lignosulfonate 4.8 % DM + carinata meal 71.4 % DM + pea screenings 23.8 % DM; BPP5: lignosulfonate 0 % DM + canola meal 50 % DM + pea screenings 50.0 % DM; BPP6: lignosulfonate 4.8 % DM + canola meal 47.6 % DM + pea screenings 47.6 % DM; BPP7: lignosulfonate 0 % DM + canola meal 75 % DM + pea screenings 25 % DM; BPP8: lignosulfonate 4.8 % DM + canola meal 71.4 % DM + pea screenings 23.8 % DM; CO-P: Co–Product. CR: Carinata meal. CN: Canola meal. LSO_3: Lignosulfonate; Baseline for protein spectral peak: ca. 1480–1730 cm^{-1}; protein amide I region: ca. 1569–1730 cm^{-1}; protein amide II region: ca. 1480–1569 cm^{-1}; center range of amide I peak: ca. 1638–1649 cm^{-1}; center range of amide II peak: ca. 1533–1540 cm^{-1}; center range for α–helix: ca. 1647–1653cm^{-1}; center range for β–sheet: ca. 1625–1631cm^{-1}

Table 3.2. Correlation between protein profile of different blend-pelleted products (BPP)* and the molecular spectral profile related to amide region.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	*β*-sheet height	α, *β* ratio
CP	r	0.55	0.11	0.40	0.70	0.16	0.58	0.39	0.44	-0.55
(g/kg DM)	*P*-value	0.03	0.69	0.13	0.01	0.56	0.02	0.14	0.08	0.03
NDICP	r	-0.06	-0.50	0.52	0.08	-0.40	-0.08	-0.33	-0.32	0.12
(g/kg CP)	*P*-value	0.82	0.05	0.04	0.77	0.13	0.77	0.21	0.23	0.66
ADICP	r	0.29	0.56	-0.36	0.18	0.51	0.32	0.53	0.55	-0.40
(g/kg CP)	*P*-value	0.27	0.02	0.17	0.49	0.04	0.23	0.03	0.03	0.13
SCP	r	0.25	0.48	-0.38	0.05	0.35	0.16	0.41	0.37	-0.12
(g/kg CP)	*P*-value	0.36	0.06	0.15	0.85	0.19	0.56	0.12	0.15	0.65
NPN	r	-0.71	-0.12	-0.56	-0.80	-0.22	-0.68	-0.61	-0.67	0.64
(g/kg CP)	*P*-value	0.01	0.67	0.02	0.01	0.42	0.01	0.01	0.01	0.01

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non–protein nitrogen.

and amide I height (r = 0.55, P = 0.03) in BPPs. However, CP exhibited a negative correlation with the ratio of α–helix to β–sheet (r = –0.55, P = 0.03).

The results in the current study showed that NDICP had a negative correlation with amide II height (r = –0.50, P = 0.05) and a positive association with the ratio of amide I to II height (r = 0.52; P = 0.04). The content of ADICP was found to be positively correlate with the amide II height (r = 0.56, P = 0.02) or the amide II area (r = 0.51, P = 0.04). The concentration of NPN was negatively correlated with the amide I area (r = –0.80, P = 0.01), amide I height (r = –0.71, P < 0.05), amide I to II area ratio (r = –0.68, P < 0.05), amide I to II height ratio (r = –0.56, P < 0.05), β-sheet height (r = –0.67, P < 0.05), and α–helix height (r = –0.61, P < 0.05). However, there was a positive correlation between NPN and the ratio of α–helix to β sheet (r = 0.64, P = 0.01).

For the truly digestible crude protein, the results showed some correlations between tdCP and amide I area (r = 0.68, P = 0.01), amide I height (r = 0.53, P < 0.05), and the amide area (r = 0.56, P = 0.02; Table 3.3). On the other hand, there was no correlation (P > 0.05) between the predicted energy values by the NRC-model and the molecular structure features related to protein amide region.

For protein subfractions partitioned by the CNCPS 6.5 model, the results showed that the slowly degradable protein (PB2 fraction) was positively associated with the amide I to II height ratio (r = 0.50, P = 0.05) and negatively related to the amide II height (r = –0.52, P = 0.04; Table 3.3). The PC fraction was observed to be positively associated with the amide II height (r = 0.56, P = 0.02) and amide II area (r = 0.51, P = 0.04).

In the current study, it has been found the degradation rate of feed protein was negatively correlated with the amide I area (r = –0.36, P < 0.01) and the amide I to II height ratio (r = –0.36,

Table 3.3. Correlation between protein subfractions and predicted energy values of different blend-pelleted products (BPP)* and the molecular spectral profile related to the amide region.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	*β*-sheet height	α, *β* ratio
CNCPS v.6.5 protein subfractions (g/kg CP)										
PA2	r	0.23	0.47	-0.39	0.04	0.34	0.15	0.40	0.36	-0.10
	P-value	0.39	0.06	0.13	0.90	0.19	0.59	0.13	0.17	0.71
PB1	r	-0.28	-0.31	0.18	-0.10	-0.20	-0.15	-0.32	-0.28	0.06
	P-value	0.29	0.24	0.50	0.70	0.46	0.58	0.22	0.29	0.83
PB2	r	-0.11	-0.52	0.50	0.03	-0.43	-0.13	-0.38	-0.37	0.18
	P-value	0.68	0.04	0.05	0.92	0.10	0.64	0.15	0.16	0.51
PC	r	0.29	0.56	-0.36	0.18	0.51	0.32	0.53	0.55	-0.40
	P-value	0.27	0.02	0.17	0.49	0.04	0.23	0.03	0.03	0.13
Predicted energy values (Mcal/kg)										
ME_{p3x}	r	-0.12	-0.43	0.36	-0.02	-0.38	-0.15	-0.38	-0.39	0.27
	P-value	0.67	0.09	0.17	0.94	0.14	0.58	0.15	0.13	0.30
NE_{Lp3x}	r	-0.12	-0.44	0.37	-0.02	-0.39	-0.15	-0.37	-0.39	0.27
	P-value	0.67	0.09	0.16	0.95	0.14	0.59	0.15	0.14	0.32

Table 3.3. *Cont'd.* Correlation between protein subfractions and predicted energy values of different blend-pelleted products (BPP)* and the molecular spectral profile related to the amide region.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	β-sheet height	α, β ratio
Predicted total digestible nutrients (g/kg DM)										
tdCP	r	0.53	0.09	0.41	0.68	0.13	0.56	0.36	0.42	-0.53
	P-value	0.03	0.75	0.12	0.01	0.62	0.02	0.17	0.11	0.03
TDN_{1x}	r	-0.38	-0.53	0.23	-0.33	-0.50	-0.43	-0.59	-0.63	0.55
	P-value	0.15	0.03	0.40	0.21	0.05	0.10	0.02	0.01	0.03

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate ; PA2: soluble true protein; PB1, insoluble true protein; PB2: fiber–bound protein; PC: indigestible protein; $ME_{p3\times}$: metabolizable energy at production level of intake (3×); $NE_{Lp3\times}$: net energy for lactation at production level of intake (3×); tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one time maintenance.

$P = 0.04$), while the slowly degradable fraction of protein was positively correlated with the amide I height, amide I area and amide area (Table 3.4). The amide II area and the ratio of amide I to II height were found to be negatively related to the EDCP of BPPs ($P < 0.05$; Table 4). Additionally, the undegradable CP fraction of BPPs was found to be negative correlated with the heights or areas of amide I and amide II. The intestinal digestion of BCP (%dIDP) and the total intestinal digestibility of CP (IADP%) were positively correlated with the amide I to II height ratio (r = 0.55, $P < 0.05$). The results showed that the α -helix to β-sheet ratio was correlated with the slowly degradable fraction of CP (r = –0.44, $P = 0.01$) and the undegradable fraction of CP (r = 0.45, $P = 0.01$).

The correlation between protein molecular structure and the predicted protein supply of BPP is shown in Table 3.5 The data showed significant correlation between AMCP and the amide II height (r = –0.53, $P < 0.01$), the amide II area (r = –0.50, $P < 0.01$), the amide area (r = –0.43, $P < 0.01$), and the α helix to β-sheet ratio (r = 0.55, $P < 0.01$). For the truly absorbable rumen undegraded protein in the small intestine, ARUP exhibited a positive correlation with the amide I to II height ratio (r = 0.62, $P < 0.01$). The MP had a positive correlation with amide I to II height ratio (r = 0.61, $P < 0.01$) and a negative correlation with the amide II height (r = –0.43, $P = 0.02$). The DPB had positive correlations with amide II height (r = 0.70, $P < 0.01$), the total amide area (r = 0.65, $P < 0.01$), amide II area (r = 0.64, $P < 0.01$), amide I height (r = 0.63, $P < 0.01$), amide I area (r = 0.57, $P < 0.01$), β-sheet height (r = 0.79, $P < 0.01$), and α helix height (r = 0.77, $P < 0.01$) but a negative correlation with α helix to β-sheet ratio (r = –0.62, $P < 0.01$). The FMV had a positive correlation with amide I to II height ratio (r = 0.45, $P = 0.01$), while negatively correlated with the amide II height (r = –0.39, $P = 0.03$).

Table 3.4. Correlation between ruminal and intestinal digestion of protein for different blend-pelleted products (BPP)* and the molecular spectral profile related to amide region.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	β-sheet height	α, β ratio
In situ ruminal degradation of CP										
Kd (%/h)	r	-0.22	0.13	-0.53	-0.36	0.12	-0.19	0.02	-0.09	0.25
	P-value	0.22	0.49	<0.01	0.04	0.53	0.30	0.90	0.62	0.17
S (%)	r	0.23	0.67	-0.65	-0.03	0.59	0.18	0.44	0.32	-0.10
	P-value	0.20	<0.01	<0.01	0.85	<0.01	0.33	0.01	0.07	0.59
D (%)	r	0.35	0.24	0.12	0.40	0.25	0.38	0.44	0.47	-0.44
	P-value	0.05	0.18	0.50	0.02	0.17	0.03	0.01	<0.01	0.01
U (%)	r	-0.40	-0.57	0.32	-0.25	-0.50	-0.36	-0.60	-0.56	0.45
	P-value	0.02	<0.01	0.07	0.16	<0.01	0.04	<0.01	<0.01	<0.01
BCP	r	0.13	-0.35	0.58	0.34	-0.29	0.15	-0.17	-0.03	-0.14
(g/ kg DM)	*P*-value	0.46	0.05	<0.01	0.06	0.11	0.40	0.36	0.88	0.45
EDCP	r	0.65	0.62	-0.20	0.58	0.53	0.68	0.75	0.77	-0.64
(g/ kg DM)	*P*-value	<0.01	<0.01	0.26	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 3.4. *Cont'd.* Correlation between ruminal and intestinal digestion of protein for different blend-pelleted products (BPP)* and the molecular spectral profile related to amide region.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	β-sheet height	α, β ratio
Intestinal digestion of CP										
dIDP	r	-0.16	-0.58	0.55	-0.04	-0.47	-0.19	-0.39	-0.38	0.16
(%)	*P*-value	0.39	<0.01	<0.01	0.85	0.01	0.30	0.03	0.03	0.38
IDP	r	-0.10	-0.56	0.59	0.07	-0.44	-0.10	-0.36	-0.33	0.07
(g/kg DM)	*P*-value	0.59	<0.01	<0.01	0.70	0.01	0.59	0.04	0.06	0.70
IDP	r	0.18	-0.34	0.61	0.36	-0.27	0.18	-0.12	0.01	-0.13
(%)	*P*-value	0.31	0.06	<0.01	0.04	0.13	0.33	0.53	0.97	0.47
Ruminal and intestinal digestion of CP										
TDP	r	0.02	-0.18	0.27	-0.02	-0.14	-0.10	0.01	-0.04	0.18
(g/kg DM)	*P*-value	0.91	0.33	0.13	0.91	0.45	0.58	0.95	0.81	0.31
TDP	r	0.61	0.09	0.43	0.74	0.10	0.62	0.37	0.51	-0.54
(g/kg CP)	*P*-value	<0.01	0.61	0.01	<0.01	0.60	<0.01	0.04	<0.01	<0.01

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; Kd: degradation rate; S: soluble fraction in the in situ incubation; D: potentially degradable fraction; U: undegradable fraction, BCP: bypass crude protein; EDCP: effectively degraded of crude protein; dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: Ruminal and intestinal digestion.

Table 3.5. Correlation between the predicted protein supply for different blend-pelleted products (BPP)* and the molecular spectral profile related to amide region

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	*β*-sheet height	α, *β* ratio
Absorbable microbial protein synthesis in the rumen (g/kg DM)										
MCP_{RDP}	r	0.64	0.68	-0.25	0.58	0.62	0.65	0.76	0.78	-0.60
	P-value	<0.01	<0.01	0.16	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
MCP_{TDN}	r	-0.38	-0.53	0.23	-0.33	-0.50	-0.43	-0.59	-0.63	0.55
	P-value	0.03	<0.01	0.22	0.06	<0.01	0.02	<0.01	<0.01	<0.01
AMCP	r	-0.38	-0.53	0.23	-0.33	-0.50	-0.43	-0.59	-0.63	0.55
	P-value	0.03	<0.01	0.22	0.06	<0.01	0.02	<0.01	<0.01	<0.01
Truly absorbable rumen undegraded protein in the small intestine (g/kg DM)										
RUP	r	0.17	-0.33	0.59	0.37	-0.24	0.19	-0.08	-0.03	-0.20
	P-value	0.35	0.07	<0.01	0.04	0.19	0.29	0.66	0.86	0.27
ARUP	r	0.11	-0.41	0.62	0.30	-0.31	0.12	-0.15	-0.11	-0.12
	P-value	0.54	0.02	<0.01	0.09	0.09	0.50	0.41	0.55	0.50
Total metabolizable protein and degraded protein balance (g/kg DM)										
MP	r	0.08	-0.43	0.61	0.27	-0.32	0.09	-0.18	-0.15	-0.09
	P-value	0.65	0.02	<0.01	0.14	0.07	0.62	0.32	0.43	0.64

Table 3.5. *Cont'd.* Correlation between the predicted protein supply for different blend-pelleted products (BPP)* and the molecular spectral profile related to amide region

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α-helix height	*β*-sheet height	α, *β* ratio
DPB	r	0.63	0.70	-0.26	0.57	0.64	0.65	0.77	0.79	-0.62
	P-value	<0.01	<0.01	0.15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Feed milk value (kg milk/ kg feed)										
FMV	r	-0.02	-0.39	0.45	0.15	-0.30	0.01	-0.25	-0.23	0.02
	P-value	0.91	0.03	0.01	0.43	0.10	0.97	0.17	0.21	0.90

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen-degraded protein; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy (discounted TDN); AMCP: truly absorbed rumen–synthesized microbial protein in the small intestine; RUP: ruminally undegraded feed CP, calculated according to the formula in NRC–2001 dairy model; ARUP: truly absorbed rumen–undegraded feed protein in the small intestine; MP: metabolizable protein (true protein that is digested postruminally and the component amino acid absorbed by the intestine); DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy–TDN available for microbial fermentation in the rumen; FMV: feed milk value (based on metabolic characteristics of protein predicted by NRC system).

3.4.3. Multiple regression analysis to choose the most important protein spectral parameters to predict protein nutritive profile and utilization in dairy cows

The multiple regressions analysis is shown in Table 3.6. The equations of protein profile showed that CP could be predicted from amide I area and α–helix height, taking 73% of the total variance. Amide I to II ratio could also be used to predict NDICP and NPN. The amide II height could be used to predict the ADICP, PB2, and PC. For truly digestible nutrients, tdCP could be predicted from amide I area and α–helix height, while the amide I height and *β*–sheet height could be used to predict TDN_{1x} with an explained total variance of 81%.

Table 3.7 shows that the amide I area and the α–helix height could be used to estimate the Kd and the undegradable fraction of CP with 46% and 62% of the total variance, respectively. The amide I height and *β*–sheet height were the best spectral variables to predict the EDCP with 67% of the variance. The amide I to II height ratio, amide area, *β*–sheet height and α helix to *β*-sheet ratio could be used to predict the intestinal digestion of CP with 90% of the variance. The results in Table 3.8 showed that the amide I to II height ratio was the best spectral feature in estimating the ARUP, MP, and FMV of BPPs, while the amide I height and *β*–sheet height would be used to estimate the DBP and AMCP.

3.1. Discussion

Recently, advanced vibrational spectroscopic techniques have been established to quantitatively estimate the primary and secondary molecular make-up of feed protein (Khan et al., 2014; Yu, 2007a). Generally, the amide I and II bands are used to detect the information about protein structure makeup and concentration (Damiran and Yu, 2011; Peng et al., 2014). However, the amide I is used more frequently than amide II to reveal the molecular structure of the protein, since the amide II originates from complex vibrations that includes numerous functional groups such as

Table 3.6. Multiple regression analysis to choose the most important protein spectral parameters to predict protein profile and energy profile.

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
Basic protein profile					
CP (g/kg DM)	Amide I area, α-helix height	Y = 4.19 + 4.12×Amide I area – 137.95 × α-helix height	0.73	2.048	<0.01
NDICP (g/kg CP)	Amide I, II ratio	Y = –17.26 + 13.27 × Amide I, II ratio	0.27	3.304	0.04
ADICP (g/kg CP)	Amide II height	Y = –2.65 + 35.84 × Amide II height	0.32	0.792	0.02
NPN (g/kg CP)	Amide I, II ratio, Amide I area	Y = 93.01 – 10.83 × Amide I, II ratio – 1.95 × Amide I area	0.78	2.081	<0.01
Predicted energy values by NRC (2001)					
tdCP (g/kg DM)	Amide I area, α-helix height	Y = 4.47 + 4.21 × Amide I area – 145.52 × α-helix height	0.73	2.137	<0.01
TDN_{1x} (g/kg DM)	Amide I height, β-sheet height	Y = 72.96 + 241.16 × Amide I height – 228.54 × β-sheet height	0.81	1.301	<0.01

Table 3.6. *Cont'd.* Multiple regression analysis to choose the most important protein spectral parameters to predict protein profile and energy profile.

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
Protein subfractions by Cornell Net Carbohydrate and Protein System					
PB2 (g/kg CP)	Amide II height	Y = 30.35 –164.28 × Amide II height	0.27	4.053	0.04
PC (g/kg CP)	Amide II height	Y = –2.65 +35.84 × Amide II height	0.32	0.792	0.02

RSD: residual standard deviation; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NPN: non–protein nitrogen; tdCP: truly digestible crude protein; $TDN_{1\times}$, total digestible nutrient at one time maintenance; PB2: fiber–bound protein; PC: indigestible protein.

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Table 3.7. Multiple regression analysis to choose the most important protein spectral parameters to predict protein ruminal digestion of crude protein (CP).

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
Degradation kinetics of CP					
Kd (%/h)	Amide I area, α–helix height	Y = 21.70 – 2.33×Amide I area – 100.29 × α–helix height	0.46	1.808	<0.01
S (%)	Amide II height, Amide II area	Y = 1.57 + 399.24 × Amide II height – 6.05 × Amide II area	0.51	2.050	<0.01
D (%)	Amide I height, *β*-sheet height	Y = 65.67 – 199.00 × Amide I height + 213.44 × *β*-sheet height	0.42	3.202	<0.01
U (%)	Amide area, α–helix height	Y = 30.82 +3.87 ×Amide area –364.64 × α–helix height	0.62	3.252	<0.01
BCP (g/kg DM)	Amide I to II ratio	Y = -132.49 + 14247 × Amide I, II ratio	0.34	28.925	<0.01
EDCP g/kg DM)	Amide I height, *β*-sheet height	Y = 125.35 – 827.29 × Amide I height – 1187.03 × *β*-sheet height	0.67	13.537	<0.01
Intestinal digestibility of CP					
dIDP (%)	Amide II height, Amide II area	Y = 102.68 –749.79× Amide II height +11.50× Amide II area	0.48	4.055	<0.01

Table 3.7. *Cont'd.* Multiple regression analysis to choose the most important protein spectral parameters to predict protein ruminal digestion of crude protein (CP).

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
IDP (%)	Amide I, II ratio, Amide area, β-sheet height, α to β-ratio	Y = 113.54 + 19.77 × Amide I, II ratio+6.73 ×Amide area –571.92 × β-sheet height–117.54 × α, β-ratio	0.90	2.149	<0.01
Total tract digestibility of CP					
TDP (g/kg DM)	Amide I area, α-helix height	Y = 42.01 + 37.43×Amide I area – 1282.98 × α-helix height	0.76	16.705	<0.01

RSD, residual standard deviation; kd: degradation rate; S: soluble fraction in the in situ incubation; D: potentially degradable fraction; U: undegradable fraction; BCP: bypass crude protein; EDCP: effectively degraded of crude protein; dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digestion of crude protein.

Table 3.8. Multiple regression analysis to choose the most important protein spectral parameters to predict protein supply using the NRC model.

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
Absorbable microbial protein synthesis in the rumen (g/kg DM)					
MCP_{RDP}	Amide I height, β-sheet height	Y = 106.55 – 703.13 × Amide I height + 1008.90 × β-sheet height	0.67	11.507	<0.01
MCP_{TDN}	Amide I height, β-sheet height	Y = 87.07 + 287.78 × Amide I height – 272.73 × β-sheet height	0.82	1.470	<0.01
AMCP	Amide I height, β-sheet height	Y = 55.72 + 184.17 × Amide I height – 174.54 × β-sheet height	0.82	0.941	<0.01
Truly absorbable rumen undegraded protein in the small intestine (g/kg DM)					
RUP	Amide I to II ratio	Y = –132.49 – 142.47×Amide I, II ratio	0.34	28.926	<0.01
ARUP	Amide I to II ratio	Y = –165. 71– 138.71×Amide I, II ratio	0.38	26.039	<0.01

Table 3.8. *Cont'd.* Multiple regression analysis to choose the most important protein spectral parameters to predict protein supply using the NRC model.

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	P-value
Total metabolizable protein and degraded protein balance (g/kg DM)					
MP	Amide I to II ratio	Y = –110.83 – 142.07×Amide I, II ratio	0.37	27.278	<0.01
DPB	Amide I height, β-sheet height	Y = 22.61 – 1166.80 × Amide I height + 1508.67 × β-sheet height	0.74	13.358	<0.01
Feed Milk Value (kg milk/ kg feed)					
FMV	Amide I to II ratio	Y = –1.62 + 2.45 × Amide I, II ratio	0.20	0.710	<0.01

RSD: residual standard deviation; MCP_{RDP}, microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen-degraded protein; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy (discounted TDN); AMCP: truly absorbed rumen–synthesized microbial protein in the small intestine; RUP: ruminally undegraded feed CP, calculated according to the formula in NRC–2001 dairy model; ARUP: truly absorbed rumen–undegraded feed protein in the small intestine; MP: metabolizable protein (true protein that is digested postruminally and the component amino acid absorbed by the intestine); DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy–TDN available for microbial fermentation in the rumen; FMV: feed milk value (based on metabolic characteristics of protein predicted by NRC 2001).

ligneous compounds (Jackson and Mantsch, 1995). Our results showed that amide I area and peak height were the highest in BPP7 and BPP3 and lowest in BPP2 and BPP6. These results are in agreement with the results obtained from wet chemistry analyses (Guevara-Oquendo et al., 2018). The high CP or amide I area values are attributed to the high inclusion level of co-product (canola or carinata meal) in those BPPs. The total amide area was highly sensitive to the changes in BPPs composition, where the amide area was increased with increasing the co-products levels or with decreasing pea screenings levels in BPPs. For example, the BPP3 or BPP7 (carinata or canola meal 75 % DM + pea screenings 25 % DM) had a higher ($P < 0.05$) total amide area than BPP1 or BPP5 (carinata or canola meal 50 % DM + pea screenings 50.0 % DM).

The ratio of amide I to II is influenced by the heat processing of feed (Doiron et al., 2009; Liu et al., 2012; Yu, 2006). Previous studies noted that the amide I to II ratio had a positive correlation with the MP of feed (Khan et al., 2014). Based on the current study results, the high amide I to II ratio of BPPs could be a consequence of the high inclusion level of co-products or adding carinata meal to BPPs. In agreement with these findings, Guevara-Oquendo et al. (2018) reported a higher indigestible protein content in the BPP based on canola meal (1.5% CP) than that of BPP based on carinata meal (3.2% CP). The low PC in BPP based on carinata meal was attributed to the higher content of NDICP and a lower content ADICP in carinata meal compared with canola meal. The NDICP is slowly degraded in the rumen and largely contributes to escaping feed protein from ruminal degradation (Russell et al., 1992). Thus, a large amount of NDICP could reach the small intestine and hence, increasing the MP supply to dairy cows (Russell et al., 1992). On the other hand, the ADICP reflects the amount of feed protein that is entirely indigestible in the gastrointestinal tract (Russell et al., 1992). Therefore, increasing the concentration of ADICP in feed could limit the total tract digestibility of protein in cattle.

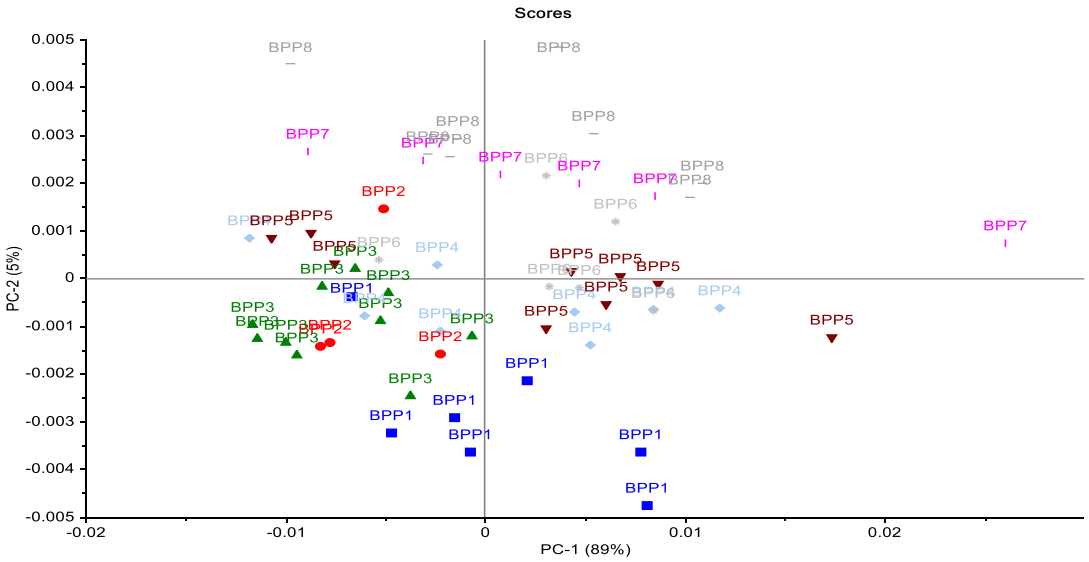
The secondary structures such as α–helix and *β*–sheet and their ratio are commonly used to detect the information about the protein's molecular makeup (Damiran and Yu, 2011; Yu and Nuez-Ortín, 2010). In the current study, all BPPs underwent the same processing. Thus it is not surprising that the ratio did not change among all BPPs. Yu (2006) and Samadi and Yu (2011) found that the ratio of α–helix to *β*–sheet was altered by the moist heating of soybean and canola seeds. The alteration in the ratio of α–helix to *β*–sheet by heat treatment was also reported by Doiron et al. (2009). The changes in the secondary structure of the protein are possibly related to the denaturation of α–helix and *β*–sheet during heat treatment. The current results showed that the ratio of α–helix to *β*–sheet decreased with decreasing the level of co-products in the BPPs, which would reflect a reduction in the MP supply in BPPs. In agreement with these results, Guevara-Oquendo et al. (2018) found that MP supply was reduced by decreasing the inclusion level of carinata or canola meal in BPPs.

The PCA procedure was used in the current study to reduce the number of variables. The PCA was performed on the molecular structure related to protein amide region (1480-1730 cm^{-1}). The first two PCs derived from the PCA classification of these spectra described 94% of the total variance in the BPPs (Figure 3.4a, b). Most of the BPPs based on canola meal such as BPP6, BPP7, and BPP8 were separated from the BPPs based on carinata meal by the PC2 which accounted for 5% of the total variance. The BPP1 exhibited the least negative values in PC2, while the BPP7 and BPP8 had the highest positive values. The PC1 which accounts for 89% of the variations among BPPs in terms of the molecular structure features did not cluster most of the BPPs. The overlapping between BPPs in the PC1 would indicate that these pellets had similar molecular structure features in the amide region.

The loading point plots were used to determine the most important areas responsible for the clustering (Figure 3.5). The amide I peak at ca. 1650 cm^{-1} was heavily loaded in PC1 and PC2, which separated the negative scores of spectra that belong to BPPs based on carinata meal from the positive score of the spectra that related to the BPP6, BPP7, and BPP8 (Figure 3.3). These findings indicate that the amide region at ca. 1650 cm^{-1} of PC2 was the most important parameters for discriminating the BPPs. These data demonstrated that the amide I peak at ca. 1650 cm^{-1} for BPPs based on carinata meal was lower than that of the BPP6, BPP7, and BPP8. These data are in agreement with the univariate analysis (Table 2) that showed BPPs based on canola meal were significantly higher in the amide I peak height compared with BPPs based on carinata meal. Based on these findings, the amide I band which is sensitive to small differentiation in molecular structure and hydrogen bonding motifs is important in the determination of protein structural and conformational changes. To obtain clear and precise peak positions of protein bands by FTIR, the raw spectra were processed by taking the second derivative (Figure 3.5a,b), which gives a negative peak for each band and shoulder in the absorption spectrum and hence allows us to identify the individual peaks among complex spectra. The PCA score plot demonstrated that the clusters of all BPPs were overlapped along PC1 (51%) and PC2 (17%). The PCA loading plots of PC1 and PC2 are shown in Figure 3.6 The loading plot showed that the positive loading could explain the variations along PC2 in the amide II region (centered at ca. 1548 cm^{-1}; N-H (60%) bending and C-N (40%) stretching vibrations: proteins a-helix), which separated the negative score of some BPPs based on canola from the positive score of BPPs based on carinata meal.

The correlation analysis between the vibrational spectral features and protein profiles, protein subfractions and the predicted energy values of BPPs. Our results for the correlation between CP and primary structure and secondary are in agreement with previous studies that

(a)



(b)

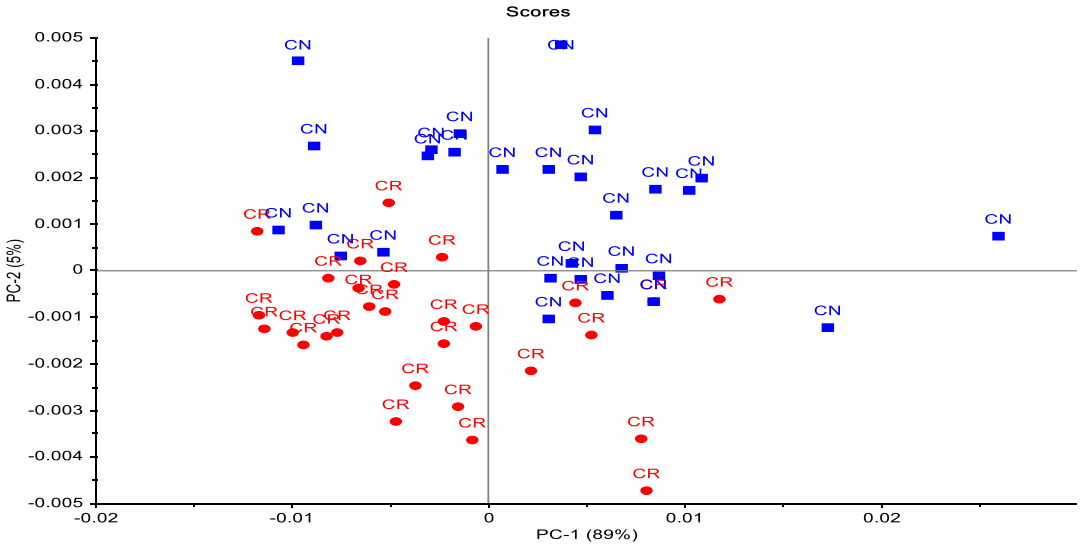
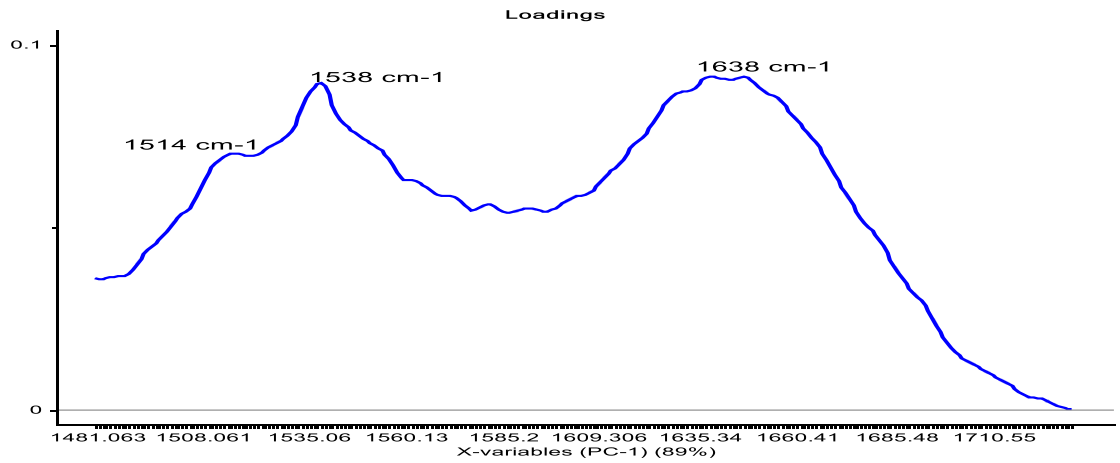


Figure 3.3. Two-dimensional score plot of the preprocessed data represents grouping of spectra along PC1 and PC2 components, describing in total 94% of variability in the blend-pelleted products a) effect of blend-pelleted products on the molecular structure changes related to protein region; b) effect of co-products on the molecular structure changes related to protein region: carinata meal (CR) vs. canola meal (CN)

(a)



(b)

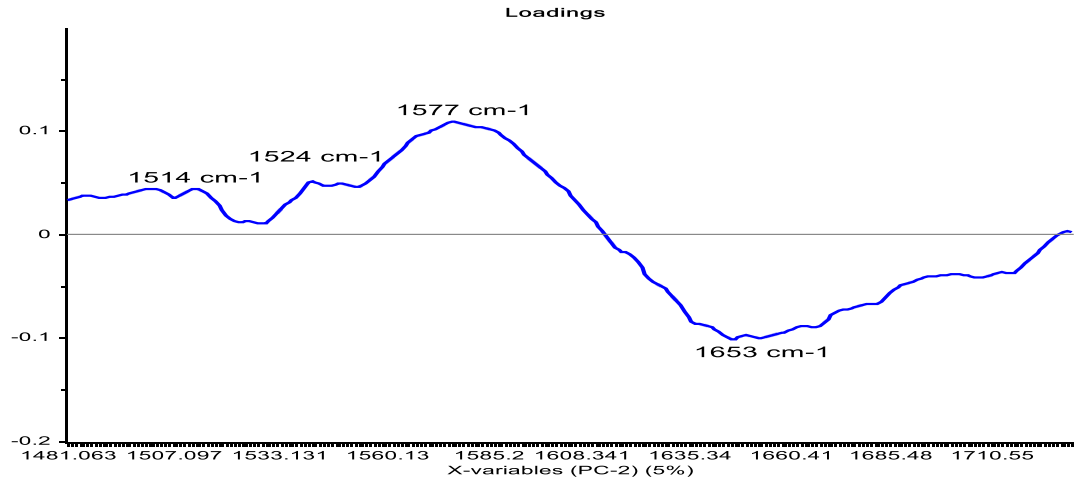


Figure 3.4. Loading plot of the first two main components (a) PC1 and (b) PC2 chosen based on the score plot of the preprocessed data

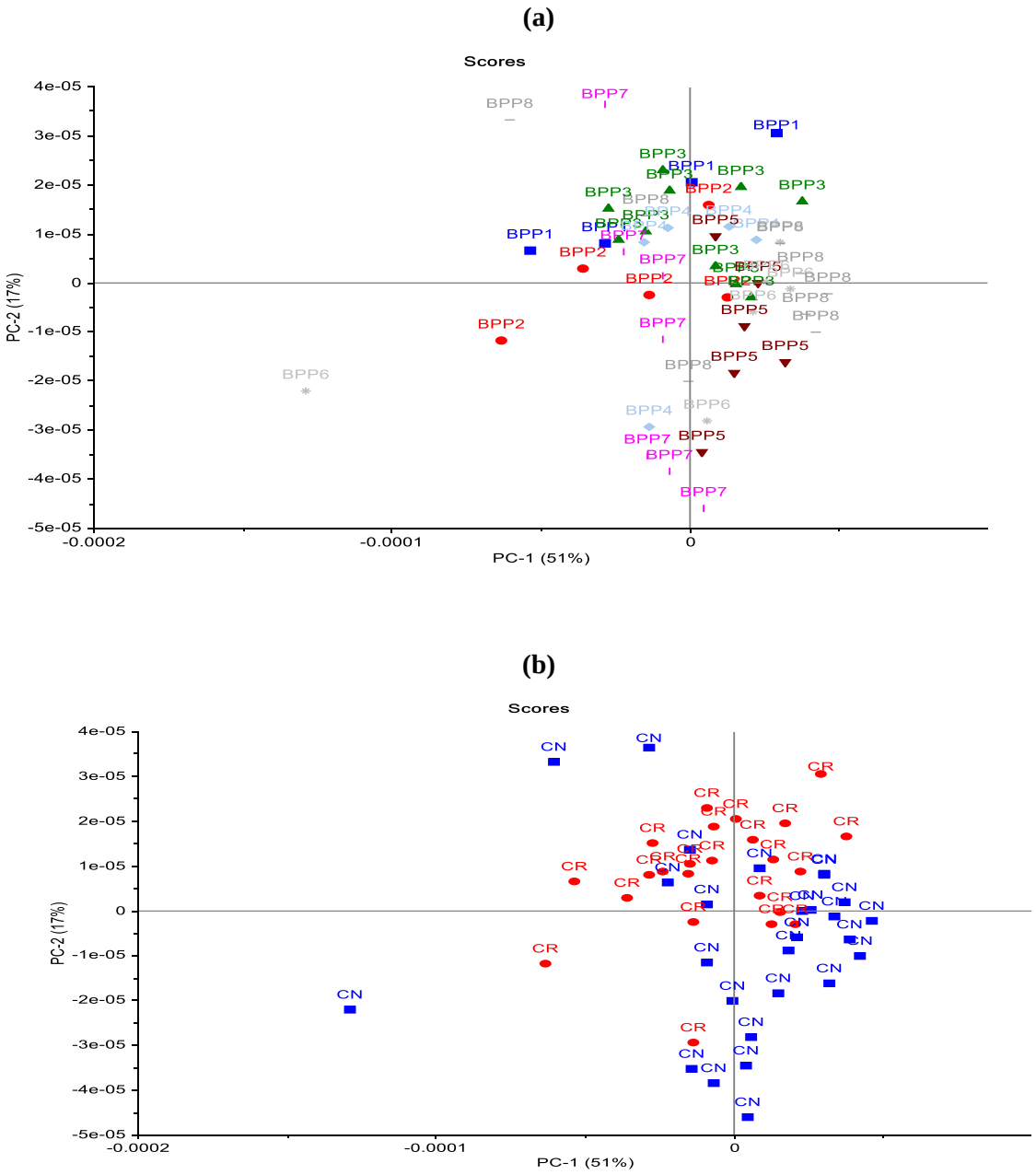
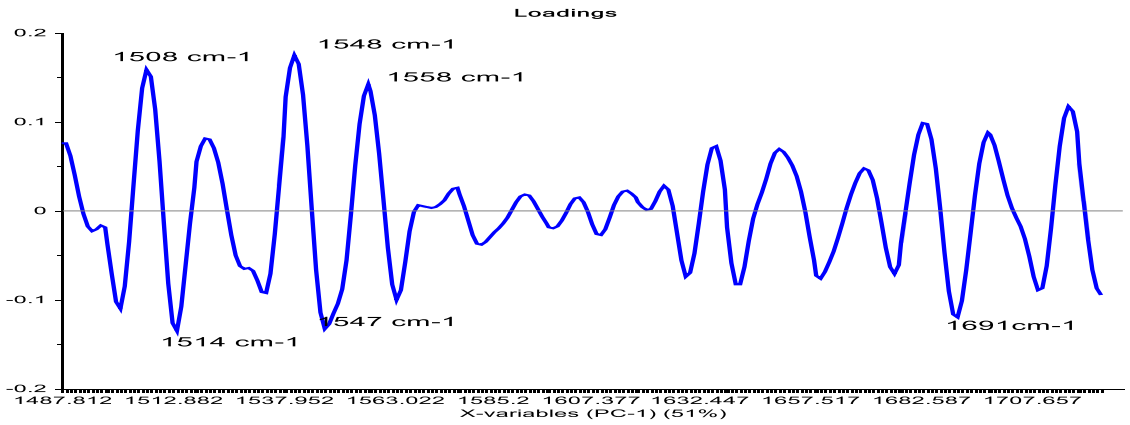


Figure 3.5. Two-dimensional score plot of the second derivative data represents grouping of spectra along PC1 and PC2 components, describing in total 68% of variability in the blend-pelleted products a) effect of blend-pelleted products (BPP) on the molecular structure changes related to protein region; b) effect of co-products on the molecular structure changes related to protein region: carinata meal (CR) vs. canola meal (CN).

reported positive correlations between CP and amide I area and amide I height of carinata meal or canola meal samples (Ban et al., 2017). Furthermore, the correlation between NDICP and primary structure is similar to the previous studies that showed NDICP had a negative association with the amide II height and a positive relationship with the ratio of amide I to II height (Yu and Nuez-Ortín, 2010). Our results showed there was no correlation ($P > 0.05$) between the predicted energy values by the NRC-model and the molecular structure features related to protein region. In line with findings, Xin and Yu (2013a) and Theodoridou and Yu (2013b) did not detect any association between the predicted energy values and the molecular structure characteristics of the protein.

For the CNCPS fractions, the current study results are in agreement with Theodoridou and Yu (2013b) who reported a positive correlation between PB2 subfraction with the amide I to II height ratio. However, there was no association between the CNCPS fractions and α-helix to *β*-sheet ratio. In agreement with observations, Huang et al. (2017) reported no correlation between α -helix to *β*-sheet ratio and the protein subfractions estimated by the CNCPS model.

For the in situ degradation parameters, a previous study noted that the ratio of amide I to II was highly correlated with the in situ rumen degradation kinetic parameters of feed protein (Yu and Nuez-Ortín, 2010). These associations are affected by the enzymatic digestion of protein in the rumen (Yu and Nuez-Ortín, 2010). It has been observed the changes in the ratio of α -helix to *β*-sheet ratio would induce alterations in the molecular protein makeup (Yu, 2007b). Heat treatment of feed was found to decrease the solubility of feed protein and increase the ADICP and NDICP in the feed as a consequence of protein denaturation during the heating process. Furthermore, the heat treatment could increase the cross-linkages among the amino acids in the polypeptide chain and reduce the sugars and finally decrease the solubility of CP (Licitra et al., 1996). Previous studies found that applying the heat treatment and increasing the heating time, caused an increase



(b)

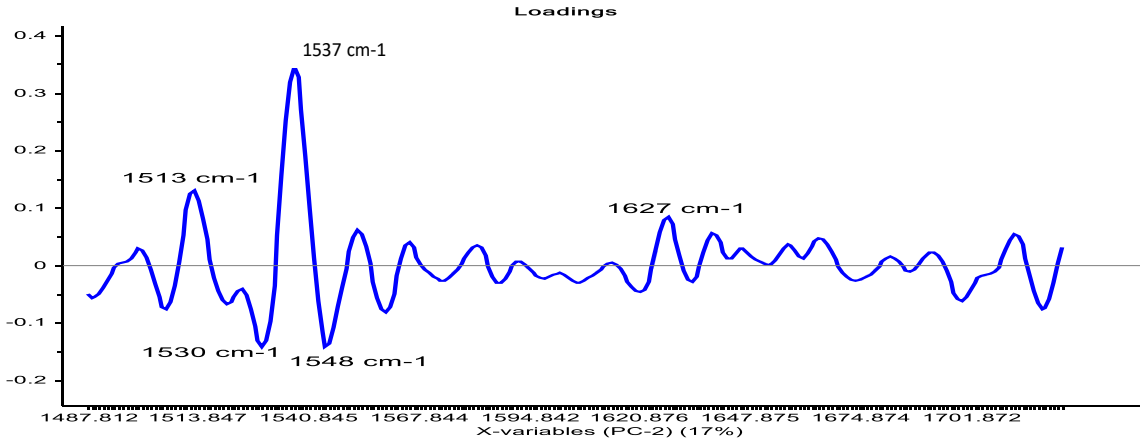


Figure 3.6. Loading plot of the first two main components (a) PC1 and (b) PC2 chosen based on the score plot of the second derivative data.

in the α–helix to *β*-sheet ratio in flaxseed and bio-ethanol co-products, respectively (Doiron et al., 2009; Yu and Nuez-Ortín, 2010). In the current study, the α -helix to *β*-sheet ratio was correlated with the slowly degradable fraction of CP (r = –0.44, *P* = 0.01) and the undegradable fraction of CP (r = 0.45, *P* = 0.01). These findings are in agreement with a previous study that found strong correlations between the α -helix to the *β*-sheet ratio of camelina seeds and the in situ ruminal degradation parameters of feed protein (Khan et al., 2015). The estimated correlation coefficient values in this study were lower than that reported by Khan et al. (2015) due to the diversity in protein origin in BPPs which applied adverse effects on the accuracy of predictions (Williams and Starkey, 1980).

3.2. Conclusions

In conclusion, the results in the current study indicated that the FTIR spectroscopy could reveal molecular structure features related to the protein of blend-pelleted products based on canola or carinata meal. The univariate analysis showed differences in absorption intensity of the functional groups related to the primary structure of the protein. The secondary structure of protein was not affected by BPP because all ingredients underwent to the same processing condition. The amide I to II height ratio was the best spectral parameter to estimate the changes in the protein degradation and the metabolizable protein of BPP.

On the basis of results in the previous chapter (Chapter 3), the blend pelleted-product based on carinata meal (BPP4) or based on canola meal (BPP8) have higher metabolizable protein supply and higher AmideI to II ratio that can exhibit a high feeding value in dairy cows, however the results of the current are based on in situ or FTIR analyses. Therefore, for my next experiments (Chapter 4, 5) I focused on evulating the blend pelleted-product based on carinata meal (BPP4) on dairy cows performance in comparison with blend pelleted-product based on conventional protein source i.e canola meal (BPP8). Also, studying the correlation between the molecular structure features of these pellets and the production performance of high producing diary cows.

4. ASSOCIATION BETWEEN MOLECULAR STRUCTURE FEATURES AND PRODUCTION EFFICIENCY OF BLEND-PELLETED PRODUCTS BASED ON BIOENERGY CO-PRODUCTS IN HIGH PRODUCING DAIRY COWS

4.1. Abstract

The main objectives of this study were: (1) to examine the effects of feeding newly-developed blend-pelleted products based on carinata meal (BPPCR) or blend-pelleted products based on canola meal (BPPCN) in combination with pea screenings and lignosulfonate on production efficiency of high producing dairy cows; (2) to determine amide molecular structure profiles changes in relation to nutrient utilization and bioavailability of BPPCR and BPPCN in dairy cows; and (3) to quantify the correlation between the molecular structural features related to the amide region and nutrient utilization in high producing dairy cows. In this study, nine mid-lactating (3 cannulated + 6 non-cannulated) Holstein cows (body weight: 679 ±124 kg; days on milk = 96 ± 22; average parities = 3) were randomly assigned to one of the following three dietary treatments: Control = control diet (common barley-based diet in western Canada); BPPCR = basal diet supplemented with 12.3 %DM BPPCR (carinata meal 71.4 % + pea screenings 23.8% + lignosulfonate 4.8 %DM), and BPPCN = basal diet supplemented with 13.3 %DM BPPCN (canola meal 71.4% + pea screenings 23.8 % + lignosulfonate 4.8%DM) in triplicated 3×3 Latin square design. Each experimental period lasted for 21 days with14 days for adaptation and seven days of sampling. The results showed that there were no differences ($P > 0.10$) among treatments in milk yield (averaging 47.5 kg/d) and FCM 3.5% (averaging 44.8 kg/d). There was no effect ($P > 0.10$) of dietary treatments on milk composition or milk component yield. The feed efficiency expressed as fat corrected milk / dry matter intake was not affected ($P > 0.10$) by the treatments (averaging 1.76). Gross milk revenue (butterfat revenue + protein revenue + other solids revenue) was similar

among the three groups (averaging $30.8 cow/day). The income over feed cost was not affected ($P > 0.10$) by all dietary treatments (averaging $23.4 cow/day). Total-tract digestibility of feed nutrients was not ($P > 0.10$) affected by different treatments. There was no effect of dietary treatments on the secondary molecular structural features of the amide region ($P > 0.10$). The correlation study showed that the amide I area and height tended to correlate with urinary N excretion (g/d) ($0.05 < P < 0.10$). Amide II area exhibited a good correlation with fecal N excretion ($r = -0.71$, $P < 0.05$) and total N excretion ($r = - 0.79$, $P < 0.05$). As to milk N, both amide I height and α to β ratio were correlated with milk N content ($P < 0.05$). Amide I height tended to correlate with the apparent N balance ($0.05 < P < 0.10$). In conclusion, the blend-pelleted products based on carinata meal as a new co-product from bio-fuel processing industry was equal to the other pelleted products based on canola meal as a protein source for dairy cattle without affecting the performance of high producing dairy cows. Molecular spectroscopy could identify structural characteristics in dietary treatments based on different bio-energy co-products. Molecular structural features related to the amide region were highly associated with the nutrient utilization in dairy cows.

4.2. Introduction

Canada produces more canola oil than any other country with about 20 million tonnes produce of canola seeds per year (Canola Council of Canada, 2018). Canola meal is a co-product of canola oil processing which is characterized by a high protein content (about 36-39% CP); furthermore, canola meal is a good source of amino acids (AAs; Canola Council of Canada, 2015).A new co-product, carinata meal, is an excellent protein source, as it contains approximately 48% CP (Xin and Yu, 2013a) and AAs (Guevara et al., 2018). Canada is a second country in the world to produce peas. Pea is a good source of protein, with approximately 24% DM and starch 46% DM (Hickling

et al., 2003). Nevertheless, to our knowledge, there is limited information that could be found in the current literature on carinata or canola meal in combination with other feedstuff to optimize its feeding value, and in particular, their, physicochemical functions. Heat and chemical treatments are the most common methods to maximize the utilization of protein and protected the AAs. It is essential to use heat treatments to improve the nutritional, chemical, hygienic, physical, and other animal feed characteristics. The heat treatment can modify the AAs residues of proteins by reacting with other compounds to decrease ruminal protein degradation. Chemical treatments, such as formaldehyde (Crooker et al., 1983), tannins (Chung et al., 2013), lignosulfonate, and xylose (McAllister et al., 1993) can also decrease rumen degradable protein in different rations.

There are many methods for feed evaluation such as wet chemistry analysis; however, this technique could damage the main structure of samples (Yu et al., 2014). The feeding value and fermentation features of feedstuff have been reported to be influenced by the inherent molecular structure (Yu, 2005). The infrared (IR) spectral region (ca. 4000-800 cm^{-1}) has a strong characteristic vibrational transition compared with the near-IR region, especially in the wavelength ranged between ca. 1800 and 800 cm^{-1}, which is called the "fingerprint region" (Liu, 2009; Yu, 2005). Vibrational spectroscopies such as FTIR is commonly used to detect the molecular structure of feed. FTIR spectroscopy is a direct, rapid, non–destructive, and non–invasive bioanalytical technique used to reveal the infrared spectrum of absorptions or emissions of liquid, gas, or solids (Smith, 2009). FTIR spectroscopy has many advantages such as explaining molecular structural changes of different types of feed and determining the nutrient utilization of feed in ruminants (Yu, 2005). Moreover, this technique could recognize the molecular structure of different crop varieties, feed ingredients, and could be used to study the effects of feed processing on protein and carbohydrate-related structures (Abeysekara et al., 2013; Huang et al., 2015; Peng et al., 2014; Xin

and Yu, 2013b). However, there is no systematic study has been conducted to determine how the BPP based on different co-products from bio-oil or bio-fuel processing (i.e., carinata or canola meal) could induce changes in protein intrinsic molecular structures and how these changes influence N-utilization in dairy cows.

The main objectives for the current study were: (1) to investigate the effects of feeding newly-developed blend-pelleted product based on new protein feed (carinata meal) or conventional protein feed (canola meal) in combination with pea screenings and lignosulfonate on production performance and economic return in high-producing dairy cows; (2) to assess the protein molecular structure profiles changes relative to nutrient bioavailability of blend-pelleted co-products in dairy cows; and (3) to estimate the correlation between molecular structure features related to amide region and the nutrient utilization in high producing dairy cows.

4.3. Materials and Methods

4.3.1. Animals and experiment design

Nine multiparous lactating Holstein cows (body weight: 679 ±124 kg; days on milk= 96 ± 22; average parities = 3) were used in a triplicated 3 × 3 Latin square design with three dietary treatments. Each experimental period lasted for 21 days, consisting of 14 days of diet adaptation and seven days of sample collection. Three cows were ruminally-cannulated cows, and they were used to determine dietary effects on ruminal fermentation. All cows were housed in individual tie-stalls at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, Canada).

4.3.2. Experimental treatments

Nine cows were randomly assigned to one of the following three dietary treatments: Control = control diet: common barley-based diet in western Canada (6.2% canola meal + 2.2 %

soybean meal + 3.9 % peas), BPPCR diet: basal diet supplemented with 12.3 %DM BPPCR (carinata meal 71.4 % + pea screenings 23.8% + lignosulfonate 4.8 %DM), and BPPCN diet = basal diet supplemented with 13.3 %DM BPPCN (canola meal 71.4% + pea screenings 23.8 % + lignosulfonate 4.8 %DM; Table 4.1). All diets were formulated using NDS Professional software based on CNCPS 6.55 (RUM&N, Italy). Carinata meal from bio-fuel processing of *Brassica carinata* seed was acquired from Agrisoma (Saskatoon, SK, Canada). Canola meal from bio-oil processing of canola seed was obtained from Cargill Animal Nutrition (Calve, SK, Canada). Pea screenings, the by-product of pulse peas processing, came from ILTA Grain Company (Surrey, BC, Canada) and lignosulfonate is a chemical compound (Ameribond) which was used as a feed additive. All the ingredients were acquired through the Canadian Feed and Research Centre (CFRC, North Battleford, SK, Canada). Minerals premix and tallow fat were added to the pellets to avoid any negative effect of minerals on palatability. For pellet processing, the different combinations were mixed in the Scott Equipment model TSM 363 (New Prague, MN, USA) for two minutes. All different mixtures were pelleted using Colorado Mill Equipment ECO-R30 (Cañon City, CO, USA) at 65°C and through a 3.6 mm diameter die. Dwelling time in the die did not exceed 15 seconds. Then, the pellets were cooled at room temperature (21°C) before collecting and storing them.

4.3.3. Sampling and data collecting

The feed intake and refusal were recorded daily before morning feeding in each period for determining the nutrients intake. From day 17 to 21, the fresh total mixed ration and refusal samples were collected and stored at -20°C for later analysis. For milk production, daily milk yield for all nine cows were recorded for each period. From day 15 to 17, milk samples were collected from all three milking's at 0630, 0230, and 2130 h into vials contained 2-bromo-2-nitropropane-

1-2-diol as a preservative. Samples were pooled per cow per day proportionally based on milk yield and submitted to the CanWest DHI Laboratory (Edmonton, AB, Canada) for CP, fat, lactose, and milk urea nitrogen (MUN) analysis using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990). Each cow was weighted at the beginning and end of each period for determining the body weight change.

Total collections of feces were conducted from 0700 h on day 18 to 0700 h on day 20 using six cows. For nitrogen balance, urine total collection was performed from day 18 until day 20. Indwelling Bardex Foley urinary bladder catheters (26 Fr, 75cc ribbed balloon, lubricious-coated; C.R. Bard Inc., Covington, GA, USA) were inserted at 0800 h on day 17. To protect the catheter inside the urinary bladder, the ribbed balloon was infused with 80 mL of double-distilled water (ddH_2O) after inserting the catheter into the urinary bladder. Urinary bladder catheters were connected to urine collection tubing on day 18 at 0700 h. Urine was acidified twice daily by adding 50 mL of 10 N sulfuric acid to the collection vessel to prevent nitrogen loss. Total daily urine was weighted and recorded (the weight of added HCl was considered negligible) and a 5% sub-sample that was pooled by the cow for each period was collected and stored at -20°C for later N analysis.

Feces were collected into large steel trays that were placed the gutter behind each tie-stall. The total daily fecal output of each cow was thoroughly mixed inside the steel tray and transferred into a pre-weighed plastic container and weighed. A 2.5% sub-sample of daily fecal production was collected and stored at -20°C for later chemical analysis and digestibility.

4.3.4. Chemical analysis of the samples

At the end of each period, total mixed ration (TMR), refusal, and fecal samples were thawed and dried at 60°C in a forced-ventilation oven, air equilibrated, (AOAC, 1990; method 930.15) and weighed to determine partial DM. Once dried, these samples were ground using a

knife mill fitted with a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Fecal samples were ground through a 1-mm screen using a Retsch ZM100 ultra centrifuge mill (Retsch-Allee 1-5, 42781 Haan, Germany). The TMR, refusal, and fecal samples were pooled per collection period for each cow. All TMR samples were analyzed for quantification of ash (AOAC, 1990; method 942.05), CP (AOAC, 1990; method 990.03), ether extract (EE; AOAC, 1990; method 2003.05), neutral detergent insoluble crude protein (NDICP), acid detergent insoluble crude protein (ADICP), and non protein nitrogen (NPN; Licitra et al., 1996), soluble crude protein (SCP; Roe et al. 1990), neutral detergent fiber (NDF; Van Soest et al., 1991 with modifications), acid detergent fiber (ADF) and lignin (ADL; AOAC, 1990; method 973.18 with modifications), carbohydrate (CHO; which was estimated as: CHO = 100 - EE - CP – ash), and non-fiber carbohydrate (NFC; which was estimated as NFC = 100 - (NDF - NDIP) - EE-CP – ash), and starch (which was analyzed using Assay Kit (Wicklow, Ireland) and by the α-amylase/amyloglucosidase method; McCleary et al., 1997). For refusal and fecal samples, DM, CP, NDF, and ADF were analyzed using the same analysis procedure. Frozen urine samples were thawed at room temperature and subsequently analyzed for total N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). For energy values, total digestible CP (tdCP), tdNFC, total digestible NDF (tdNDF), total digestible fat (tdFA), total digestible nutrients at a maintenance level (TDN_{1x}), digestible energy at 3x maintenance level (DE_{3x}), metabolizable energy at 3x maintenance level (ME_{3x}), and net energy of lacation at 3x maintenance level (NEL_{3x}) were estimated using NRC (2001) dairy.

The protein and carbohydrate subtractions were calculated using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5. For protein fractions, PA2 was estimated using the following equation: PA2 = SP × CP/100 and its Kd range was 10-40 %/h, PB1 was estimated using the

following equation: PB1 = CP − (PA2 − PB2 − PC) and its Kd range was 3-20 %/h, PB2 was equal to (NDICP − ADICP) × CP / 100) and its Kd range was 1-18 %/h, and PC fraction as indigestible protein evaluated as PC = ADICP × CP / 100. For carbohydrate fractions, CA4 and its Kd range was 40-60 %/h; CB1 and its Kd range was 20-40 %/h, fraction CB2 was estimated using the following equation: CB2 = NFC − CA4 − CB1 and its Kd range was 20-40 %/h, CB3 fraction evaluated using the following equation: CB3 = aNDFom – CC; and CC fraction evaluated as CC = (aNDFom × (Lignin × aNDFom) × 2.4)/100.

4.3.5. Collecting spectra related to the primary and secondary structural components of the amide region

For the molecular structure analysis, the TMR samples were grounded to pass a 0.12 mm sieve (Retsch ZM200, Rose, Scientific Ltd., Canada) for FTIR spectroscopic analysis. All feed samples were analyzed as described in Chapter 3. The spectral features related to the amide region were analyzed using the univariate approach.

4.3.6. Statistical analysis

Production data from all 9 cows were analyzed by Proc Mixed procedure of SAS (SAS version 9.4; SAS INC, CARY, NC, USA) as a triplicated 3 × 3 Latin square design using the following model: $Y_{ijkl} = \mu + S_i + P_{j(i)} + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$, where, Y_{ijkl} was the dependent variable, μ was the overall mean, S_i was the fixed effect of i^{th} square, $P_{j(i)}$ was the fixed effect of j^{th} period (within square i), $C_{k(i)}$ was the random effect of k^{th} cow (within square i), T_l was the fixed effect of l^{th} dietary treatment, ST_{il} was the interaction between i^{th} square and l^{th}, and E_{ijkl} was the residual error.

Apparent ruminal, nitrogen balance and total-tract nutrient digestibility were analyzed using the Proc Mixed procedure of SAS as a 3 × 3 Latin square design according to the following model: $Y_{jkl} = \mu + S_i + P_j + C_k + T_l + E_{jkl}$ where, Y_{jkl} was the dependent variable, μ was the overall mean, P_j was the fixed effect of the *j*th period, C_k was the random effect of the k^{th} cow, T_l was the fixed effect of the *l*th treatment, S_i was the fixed effect of i^{th} square, and E_{jkl} was the residual error. The Kenward-Roger method was used to approximate degrees of freedom. Multi-treatment comparison was carried out by using Tukey method. Significance was declared at $P < 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

The data of functional groups in amide region (ca. 1480–1730 cm^{-1}), chemical profile, CNCPS, energy values were analyzed by SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The experimental data were analyzed using a completely randomized design.

The correlation between the functional groups related to protein region (amide I, II peak highest and areas, α–helix, *β*–sheet and their ratio) and the chemical profiles of protein, energy values, and nitrogen balance was analyzed by using the PROC CORR procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Rank correlation with the SPEARMAN option and normality test with the UNIVARIATE option were used in the correlation study.

Multiple regression analysis (with model variable selection method) was performed to select the best functional groups that would explain the nutritive values of BPP using the PROC REG procedure of SAS with a reversed stepwise option. The following model was used for the multiple regression with model variable selection: model Y= spectral parameter 1 + spectral parameter 2 + spectral parameter 3 + spectral parameter 4 + ... + error. The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". All variables left in the final prediction models were significant at the 0.05 level. Residual analysis

was performed to check model assumptions and normality was tested using the Univariate procedure of SAS with Normal and Plot options.

4.4. Results

4.4.1. Dietary characteristics

The control diet is an example of a commercial diet that is commonly used in the western Canada, and which contains barley grain as an energy source a blend of canola meal and soybean meal as a source of N, while peas are used as a pelleting agent (Table 4.1). For the other dietary treatments, BPPCR diet was supplemented with 12.3 %DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4% + pea screenings 23.8 % + lignosulfonate 4.8 %DM) and BPPCN was supplemented with 13.3% blend-pelleted products based on canola meal (BPPCN: canola meal 71.4 % + pea screenings 23.8 % + lignosulfonate 4.8 %DM).

4.4.2. Chemical profiles

Table 4.2 shows the chemical profiles for the different dietary treatments (n = 3). There was no significant ($P > 0.10$) difference among the different diets in CP and EE contents. Furthermore, there was no effect ($P > 0.10$) of the three dietary treatments on protein profiles (SCP, ADICP, and NDICP) and carbohydrate profiles (CHO, NDF, ADF, and starch). However, the lignin content expressed as ADL was significantly lower ($P = 0.01$) in the BPPCN diet (3.5 %DM) compared with BPPCR diet and control diet (averaging 4.1%DM).

Table 4.1 Chemical composition and ingredient of total mixed ration for the supplement diet treatments

Items	Dietary treatments		
	Control	BPPCR	BPPCN
Ingredient (%DM)			
Barley silage	38.0	38.0	38.0
Alfalfa hay	16.0	16.0	16.0
Barley grain	30.0	30.0	29.0
Canola meal	6.2	-	-
Soybean meal	2.2	-	-
Peas	3.9	-	-
BPPCR	-	12.3	-
BPPCN	-	-	13.3
Potassium magnesium sulfate	0.2	0.2	0.2
Sodium bicarbonate	0.5	0.5	0.5
Tallow	0.8	0.8	0.8
Limestone ground	0.1	0.1	0.1
Mineral Premix*	1.0	1.0	1.0
Ameribond (Lignosulfonate)	0.2	0.2	0.2
Palmitic acid	0.9	0.9	0.9

Control diet: common barley-based diet in western Canada; BPPCR: basal diet supplemented with 12.3 %DM blend-pelleted products based on carinata meal (BPPCR: carinata meal 71.4 % + pea screenings 23.8% + lignosulfonate 4.8 %DM); BPPCN: basal diet supplemented with 13.3% blend-pelleted products based on carinata meal (BPPCN: canola meal 71.4% + pea screenings 23.8 % + lignosulfonate 4.8%DM); Composition of the premix: Calcium= 16%; Phosphorus= 8.0%; Chloride= 10.4%; Sodium= 7.6%; Potassium= 1.8%; Sulfur=1.0%; Magnesium= 4.5%; Copper= 535 ppm; Zinc= 2100 ppm; Manganese= 1500 ppm; Iron= max 1050 ppm; Selenium= 16 ppm; Iodine = 45 ppm; Cobalt= 16 ppm; Vitamin A (KIU) = 330; Vitamin D (KIU) = 60; Vitamin E (IU) = 2500.

Table 4.2 Chemical profiles for total mixed rations (TMR) with blend-pelleted products* in lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control Vs. (BPPCR + BPPCN)
Basic chemical profile of TMR						
Ash (%DM)	6.83	6.90	6.65	0.227	0.74	0.74
EE (%DM)	3.93	4.34	4.44	0.187	0.20	0.23
Protein profile						
CP (%DM)	15.87	15.77	16.20	0.188	0.30	0.15
SCP (%CP)	40.99	38.32	39.97	1.307	0.40	0.23
SCP (%DM)	6.50	6.30	6.20	0.149	0.41	0.32
ADIP (%CP)	7.35	6.93	7.18	0.309	0.65	0.93
ADIP (%DM)	1.17	1.09	1.16	0.054	0.59	0.63
NDIP (%CP)	8.22	7.84	8.11	0.372	0.77	0.87
NDIP (%DM)	1.30	1.24	1.31	0.061	0.65	0.58

Table 4.2 *Cont'd.* Chemical profiles for total mixed rations (TMR) with blend-pelleted products* in lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Carbohydrates profile						
CHO (%DM)	73.37	72.99	72.70	0.320	0.40	0.27
NDF (%DM)	29.43	29.37	29.53	0.737	0.99	0.89
ADF (%DM)	17.43	17.90	17.63	0.517	0.82	1.00
Starch (%DM)	25.87	24.27	24.07	0.690	0.21	0.28
NFC (%DM)	45.24	44.86	44.48	0.638	0.72	0.50
NFC (%CHO)	61.66	61.46	61.19	0.954	0.94	0.77
Lignin contents						
ADL (%DM)	4.00[a]	3.54[b]	4.09[a]	0.094	0.01	0.03
ADL (%NDF)	13.60[a]	12.06[b]	13.88[a]	0.382	0.03	0.07

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM = standard error of mean; [a-b] Means with different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using Tukey method; DM: dry matter; EE: ether extract; CP: crude protein; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; CHO: carbohydrate (CHO = 100 − EE − CP – ash); NDF: neutral detergent fiber; ADF: acid detergent fiber, non-structural carbohydrate; ADL: acid detergent lignin; ADL: NFC, non-fiber CHO [NFC = 100 − (NDF − NDICP) – EE − CP − ash].

4.4.3. Protein and carbohydrate subfractions

Protein and CHO were sub-fractioned using Cornell Net Carbohydrate and Protein System (CNCPS v.6.5). All protein subfractions were not affected ($P > 0.10$) by three dietary treatments (Table 4.3). However, when the protein fractions expressed as true protein, PA2 tended to be higher in BPPCN diet (61 %TP) compared with the control and BPPCR diets (averaging 56 %TP). On the other hand, there was no effect ($P > 0.10$) of different diets on CHO subfractions.

4.4.4. Protein FTIR spectral of the experimental diets

The amide spectral profile including the primary structures of Amide I and Amide II and the secondary structure of Amide I (i.e., α-helix and β-sheet) for different diets are presented in Table 4.4. The BPPCN diet had higher ($P = 0.02$) Amide I peak height (0.14 IU) compared with control diet and BPPCR diet (averaging 0.13 IU). There was no effect of dietary treatments ($P > 0.10$) on Amide II peak height. The ratio of Amide I to II height was significantly ($P = 0.04$) higher in BPPCN diet (2.73 IU) than control and BPPCR diets (averaging 2.38 IU). Amide I area was significantly higher ($P = 0.01$) in BPPCN diet (11.7 IU) compared with control diet and BPPCR diet (averaging 9.8 IU). However, Amide II area was not affected ($P > 0.10$) by different diets. There was no effect ($P > 0.10$) of different diets on the secondary structure of Amide I (α–helix and β–sheet) and their ratio.

4.4.5. Energy value

The dairy model (NRC 2001) was used to estimate the energy values for the different experimental diets (Table 4.5). Total digestible NFC, CP, NDF, and FA were not affected ($P > 0.10$) by dietary treatments. Furthermore, there were no significant ($P > 0.10$) differences among

Table 4.3 Protein and carbohydrate subfractions of the total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows in the rumen using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Protein subfractions						
PA2 (%DM)	6.30	6.35	6.00	0.064	0.24	0.16
PB1 (%DM)	8.36	8.11	9.31	0.108	0.12	0.08
PB2 (%DM)	0.12	0.12	0.06	0.070	0.85	0.64
PC (%DM)	1.02	1.08	1.23	0.089	0.54	0.36
True protein (%CP)	42.63[ab]	43.58[a]	39.04[b]	0.133	0.04	0.03
PA2 (%TP)	56.56	55.63	60.57	0.354	0.10	0.07
PB1 (%TP)	0.81	0.79	0.39	0.487	0.84	0.63
PB2 (%TP)	0.94	0.98	1.00	0.266	0.98	0.88
Carbohydrates subfractions						
CA4 (%DM)	4.80	5.45	5.90	0.447	0.53	0.41
CB1 (%DM)	26.70	23.95	25.30	0.574	0.27	0.98
CB2 (%DM)	11.98	14.01	12.57	0.798	0.44	0.75
CB3 (%DM)	25.29	26.18	24.37	0.447	0.31	0.26
CC (%DM)	4.01	3.62	3.83	0.064	0.21	0.89

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM = standard error of mean; [a-b] Means with different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using the Tukey method; PA2: soluble true protein; PB1: insoluble true protein; PB2: fiber-bound protein; PC: indigestible protein; CA4: sugars; CB1: starch; CB2: soluble fibers; CB3: digestible fiber; CC: indigestible fiber.

Table 4.4 Protein spectral profile of total mixed ration with blend-pelleted products (BPP)[*] using FTIR

Items	Treatments			SEM	*P*-value	Contrast *P*-value Control vs. (BPPCR + BPPCN)
	Control	BPPCR	BPPCN			
Protein primary structure[3]						
Amide I peak height	0.13[b]	0.13[b]	0.14[a]	0.006	0.02	0.01
Amide II peak height	0.06	0.06	0.05	0.003	0.70	0.42
Amide I, II height ratio	2.38[b]	2.38[b]	2.73[a]	0.193	0.04	0.01
Amide I area	9.71[b]	9.96[b]	11.70[a]	0.724	0.01	0.01
Amide II area	1.78	1.76	1.60	0.079	0.22	0.09
Amide area	11.49[b]	11.72[b]	13.30[a]	0.741	0.02	0.01
Amide I (%amide area)	84.43[b]	84.89[b]	87.62[a]	0.867	0.01	0.01
Protein secondary structure						
α-helix height	0.10	0.10	0.10	0.033	0.86	0.59
β-sheet height	0.11	0.11	0.10	0.033	0.89	0.64
α-helix, β-sheet height ratio	0.98	0.95	0.97	0.023	0.65	0.95

[*]Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; Baseline for protein spectral peak: ca. 1480–1730 cm^{-1}; protein amide I region: ca. 1569–1730 cm^{-1}; protein amide II region: ca. 1480–1569 cm^{-1}; center range of amide I peak: ca. 1638–1649 cm^{-1}; center range of amide II peak: ca. 1533–1540 cm^{-1}; center range for α–helix: ca. 1647–1653cm^{-1}; center range for β–sheet: ca. 1625–1631cm^{-1}.

Table 4.5 Energy value of total mixed ration with blend-pelleted products (BPP)* for lactating dairy cows**.**

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Truly digestible nutrients (%DM)						
tdNFC	44.33	43.96	43.59	0.625	0.72	0.50
tdCP	14.53	14.51	14.86	0.165	0.30	0.14
tdNDF	14.04	14.64	13.98	0.464	0.56	0.55
tdFA	2.93	3.34	3.44	0.187	0.20	0.23
Total digestible nutrients (%DM)						
TDN_{1x}	72.50	73.63	73.18	0.389	0.20	0.82
TDN_{3x}	66.58	67.62	67.21	0.358	0.20	0.81
Predicted energy values (Mcal/kg day)						
DE_{1x}	3.24	3.29	3.27	0.017	0.20	0.64
DE_{p3x}	2.97	3.02	3.00	0.016	0.24	0.69
ME_{p3x}	2.56	2.61	2.59	0.016	0.24	0.69
NEL_{p3x}	1.61	1.65	1.64	0.013	0.25	0.69

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM = standard error of mean; tdNFC: truly digestible non-fiber carbohydrates; tdCP: total digestible crude protein; tdNDF: total digestible neutral detergent fiber; tdFA: total digestible fatty acid; TDN_{1x}: total digestible nutrients; TDN_{1x}: total digestible nutrients at a production level (3x maintenance); DE_{1x}: digestible energy; DE_{p3x}: digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NEL_{p3x}: net energy at a production level (3x maintenance).

different diets in TDN at the maintenance level or at the production level. Similarly, all the predicted energy values, i.e, digestible energy, metabolizable energy, and net energy, were not affected ($P > 0.10$) by the three different diets.

4.4.6. Nutrient intakes and apparent nutrients digestibility

All dietary treatments did not affect ($P > 0.10$) DM, CP, and ADF intakes or digestibility (Table 4.6). Starch intake tended to increase ($P = 0.09$) when cows fed control diet were compared with other diets. Nevertheless, apparent starch digestibility, when expressed as a percentage of total starch intake was not affected ($P > 0.10$) by dietary treatments. Cows fed control die, or BPPCN tended to have low NDF intake ($P = 0.08$) compared with the BPPCR diet. However, apparent NDF digestibility when expressed as a percentage of total NDF intake was not affected ($P > 0.10$) by different diets.

4.4.7. Apparent nitrogen balance

There was no effect of diets ($P > 0.10$) on urinary excretion, fecal excretion, and total N excretion either expressed as a percentage of N intake or as grams per day (Table 4.7). Milk N secretion expressed as a percentage of N intake or grams per day was not similary affected ($P > 0.10$) by the different diets.

4.4.8. Milk Production and Composition

The results presented in Table 4.8 on milk production and composition are from all nine cows that were used in the study. Milk yield, fat corrected milk (FCM), and energy corrected milk (ECM) were not affected ($P > 0.10$) by the different diets. Our results showed that milk fat, milk protein, and SNF were not affected ($P > 0.10$) by any of the experimental diets. Milk urea nitrogen (MUN) was not affected ($P > 0.10$) by diets. There was no effect ($P > 0.10$) of the experimental

Table 4.6 Feed intake and total-tract nutrient digestibility for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Dry matter						
Intake (kg/d)	27.55	25.90	27.04	0.868	0.45	0.77
Apparent digestion (% of intake)	72.30	71.93	71.17	1.305	0.56	0.81
Starch						
Intake (kg/d)	7.26	6.46	6.63	0.240	0.09	0.44
Apparent digestion (% of intake)	97.28	97.06	96.97	0.544	0.93	0.79
Crude protein						
Intake (kg/d)	4.35	4.06	4.38	0.147	0.33	0.37
Apparent digestion (% of intake)	71.16	72.60	70.81	2.192	0.88	0.76
Neutral detergent fiber						
Intake (kg/d)	7.98	7.46	7.84	0.243	0.08	0.46
Apparent digestion (% of intake)	47.52	46.65	45.96	1.850	0.83	0.63

Table 4.6 *cont'd.* Total tract nutrient digestibility for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Acid detergent fiber						
Intake (kg/d)	4.66	4.51	4.70	0.169	0.64	0.48
Apparent digestion (% of intake)	41.33	41.79	38.60	2.840	0.69	0.41

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean.

Table 4.7 The apparent nitrogen balance for high producing dairy cows fed total mixed ration blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
N intake (g/d)	696.33	652.92	699.77	21.411	0.27	0.36
Urinary Excretion						
Total (kg/d)	19.91	20.79	20.22	1.843	0.94	0.95
Total N (g/d)	188.42	183.15	187.43	7.662	0.88	0.86
Total N (% of N intake)	27.45	28.03	26.84	1.399	0.75	0.52
Fecal Excretion						
N (g/d)	200.57	187.03	201.93	11.568	0.62	0.58
N (% of N intake)	27.79	30.67	28.27	1.481	0.45	0.65
Total N Excretion						
N (g/d)	388.95	370.18	389.38	3.111	0.52	0.55
N (% of N intake)	56.03	57.02	56.02	2.374	0.94	0.86

Table 4.7 *cont'd.* The apparent nitrogen balance for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Milk N						
N (g/d)	297.68	275.37	263.25	19.570	0.48	0.35
N (% of N intake)	43.09	42.27	37.55	2.643	0.36	0.17
Apparent N balance (g/d)	9.70	7.37	47.13	25.008	0.48	0.24
Productive N (g/d)	307.38	282.72	310.40	22.044	0.64	0.58
Milk nitrogen/nitrogen intake	43.12	42.25	37.55	2.535	0.29	0.13

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean.

Table 4.8 Milk yield and milk compositions in lactating dairy cows fed total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Milk yield (kg/d)						
Milk	47.81	47.36	47.46	1.561	0.79	0.84
3.5% FCM	44.78	45.37	44.24	1.601	0.78	0.73
ECM	45.33	45.44	44.67	1.475	0.20	0.21
Milk component yield (kg/d)						
Fat	1.51	1.55	1.49	0.112	0.32	0.28
Protein	1.48	1.44	1.44	0.051	0.26	0.41
Lactose	2.16	2.14	2.13	0.086	0.81	0.57
SNF	4.10	4.05	4.03	0.131	0.60	0.41
Milk composition						
Fat (%)	3.22	3.29	3.16	0.161	0.72	0.50
Protein (%)	3.09	3.05	3.05	0.063	0.33	0.43
Lactose (%)	4.50	4.53	4.49	0.045	0.79	0.59
Total solids (%)	11.80	11.83	11.66	0.225	0.49	0.25
SNF (%)	8.59	8.53	8.52	0.098	0.36	0.51
MUN (mg/L)	10.47	10.13	9.53	0.535	0.46	0.25
Efficiency						
ECM/DMI	1.77	1.80	1.73	0.049	0.26	0.11
FCM/DMI	1.73	1.79	1.70	0.050	0.19	0.19

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM = standard error of mean; FCM = Fat-corrected milk calculated as: 0.35*M + 18.57*F; where M = quantity of milk in kg, F = amount of fat in kg 'M' quantity of milk; ECM = Energy-corrected milk calculated as: =0.3246*kg of milk+12.86*kg of milk fat+7.04*kg of milk protein; SNF= Solids-not-fat; MUN = milk urea nitrogen.

diets on the efficiency when it was expressed as FCM/DMI and ECM/DMI. Additionally, there was no significant effect ($P > 0.10$) of the different diets on body weight gain and estimated net energy values (Table 4.9).

4.4.9. Economic analysis

The economic revenue of milk components is presented in Table 4.10. Results in the current study showed that the butterfat revenue, protein revenue, and other solids revenue were not affected ($P > 0.10$) by the different diets. Also, income over feed cost was not affected ($P > 0.10$) by the different diets.

4.4.10. Correlation analysis between amide spectral features and protein profiles and apparent nitrogen balance

Table 4.11 shows that CP had a positive correlation with Amide I to II height ratio (r = 0.68, $P = 0.04$) and tended to have a positive correlation with Amide I height (r = 0.62, $P = 0.08$). The SCP expressed as DM had a negative correlation with Amide I height (r = -0.73, $P = 0.03$), Amide I area (r = -0.70, $P= 0.03$), and total Amide area (r = -0.68, $P = 0.04$).

For the predicted energy, tdCP tended to have a positive correlation with Amide I height (r = 0.62, $P = 0.08$; Table 4.12). The DE_{p3x} and ME_{p3x} tended to have a negative correlation with α–helix height (r = -0.59, $P= 0.09$) and β–sheet height (r = -0.63, $P = 0.07$). The NEL_{p3x} tended to have a negative correlation with β–sheet height (r = -0.60, $P= 0.09$).

For protein subfractions, PA2 had a negative correlation with Amide I height (r = -0.73, $P = 0.03$) and Amide I area (r = -0.70, $P = 0.03$; Table 4.13). The PB1 had a positive correlation with Amide I height (r = 0.68, $P= 0.04$) and Amide I to II height ratio (r = 0.69, $P = 0.04$), and tended to have a positive correlation with Amide I area (r = 0.64, $P = 0.06$) and total Amide area (r = 0.61,

Table 4.9 Effect of different total mixed ration with blend-pellet products (BPP)* body weight gain and estimated energy values of lactating dairy cows

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
BW gain (kg/d)	0.40	-0.25	0.79	0.464	0.30	0.22
Calculated net energy values (Mcal/d)						
Milk	30.86	30.98	30.34	1.053	0.73	0.44
BW gain + Milk	32.86	29.82	34.31	2.444	0.44	0.34
Total	43.63	40.57	45.10	2.465	0.43	0.34
NE	1.70	1.60	1.76	0.113	0.61	0.44

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; BW: Body Weight; Calculated NEL: calculated total net energy: Mcal d^{-1}/ DMI (kg/d).

Table 4.10 The economic revenue of component production efficiency for total mixed rations with blend-pelleted products (BPP)* in lactating dairy cows

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Milk component revenue ($)						
Butter fat revenue	23.77	24.40	23.42	1.107	0.73	0.54
Protein revenue	3.24	3.31	3.29	0.170	0.92	0.94
Other solids revenue	3.58	3.64	3.63	0.019	0.29	0.50
Revenue/cow/day ($)	30.73	31.29	30.27	1.215	0.62	0.83
IOFC ($)	23.36	23.96	22.89	1.183	0.70	0.50

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; IFOC: Income after purchased feed cost; Income efficiency: $ income/unit DM fed.

Table 4.11. Correlation between the basic chemical profile of protein for total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows and protein molecular structure

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
Basic Protein Profile										
CP	r	0.62	-0.33	0.68	0.56	-0.37	0.53	-0.58	-0.56	-0.10
(%DM)	*P*–value	0.08	0.39	0.04	0.11	0.33	0.14	0.10	0.12	0.79
SCP	r	-0.75	0.10	-0.64	-0.71	0.34	-0.68	0.39	0.34	0.27
(%CP)	*P*–value	0.02	0.79	0.07	0.03	0.37	0.04	0.29	0.37	0.49
SCP	r	-0.73	-0.03	-0.54	-0.70	0.29	-0.68	0.25	0.18	0.32
(%DM)	*P*–value	0.03	0.94	0.14	0.03	0.46	0.04	0.52	0.64	0.40
ADICP	r	0.12	0.06	0.09	0.07	0.33	0.10	-0.47	-0.46	-0.27
(%CP)	*P*–value	0.76	0.87	0.82	0.86	0.39	0.80	0.21	0.21	0.49
ADICP	r	0.28	-0.04	0.27	0.22	0.18	0.24	-0.58	-0.57	-0.26
(%DM)	*P*–value	0.47	0.92	0.48	0.57	0.64	0.53	0.10	0.11	0.49
NDICP	r	0.15	0.56	-0.20	0.13	0.45	0.17	-0.03	-0.02	-0.30
(%CP)	*P*–value	0.70	0.12	0.61	0.74	0.23	0.65	0.95	0.96	0.43
NDICP	r	0.32	0.44	0.01	0.29	0.31	0.32	-0.19	-0.17	-0.32
(%DM)	*P*–value	0.40	0.24	0.98	0.45	0.41	0.40	0.63	0.65	0.41

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; CP: crude protein; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein.

Table 4.12. Correlation between predicted energy profiles and feed milk value for total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows and protein molecular structure

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
tdCP	r	0.62	-0.36	0.69	0.58	-0.47	0.53	-0.48	-0.45	-0.04
(%DM)	*P*–value	0.08	0.34	0.04	0.11	0.20	0.14	0.19	0.22	0.92
TDN_1x	r	0.12	-0.21	0.22	0.16	-0.44	0.12	-0.50	-0.54	0.26
(%DM)	*P*–value	0.76	0.58	0.58	0.67	0.23	0.76	0.17	0.13	0.50
DE_1x	r	0.18	-0.24	0.28	0.23	-0.50	0.18	-0.52	-0.55	0.25
(%DM)	*P*–value	0.65	0.54	0.46	0.55	0.17	0.64	0.15	0.12	0.52
TDN_3x	r	0.12	-0.21	0.21	0.17	-0.44	0.12	-0.50	-0.54	0.26
(%DM)	*P*–value	0.76	0.59	0.58	0.67	0.23	0.76	0.17	0.14	0.50
DE_{p3x}	r	0.21	-0.28	0.34	0.26	-0.50	0.21	-0.59	-0.63	0.20
(%DM)	*P*–value	0.59	0.46	0.37	0.50	0.17	0.59	0.09	0.07	0.61
ME_{p3x}	r	0.21	-0.28	0.34	0.26	-0.50	0.21	-0.59	-0.63	0.20
(%DM)	*P*–value	0.59	0.46	0.37	0.50	0.17	0.59	0.09	0.07	0.61
$NEL_{p3x4.}$	r	0.14	-0.29	0.29	0.20	-0.51	0.14	-0.55	-0.60	0.26
(%DM)	*P*–value	0.71	0.45	0.45	0.61	0.16	0.71	0.12	0.09	0.50

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one times maintenance. $DE_{1\times}$: digestible energy at production level of intake; TDN_{3x}: total digestible nutrients at production level of intake (3×); $DE_{P3\times}$: digestible energy at production level of intake (3×); $ME_{p3\times}$: metabolizable energy at production level of intake (3×); $NEL_{P3\times}$: net energy for lactation at production level of intake (3×).

Table 4.13. Correlation between protein subfractions of protein of total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows in the rumen and intestine using the Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5 and protein molecular structure.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
PA2 (%DM)	r	-0.73	-0.03	-0.54	-0.70	0.29	-0.68	0.25	0.18	0.32
	P–value	0.03	0.94	0.14	0.03	0.46	0.04	0.52	0.64	0.40
PB1 (%DM)	r	0.68	-0.27	0.69	0.64	-0.43	0.61	-0.46	-0.41	-0.16
	P–value	0.04	0.47	0.04	0.06	0.25	0.08	0.22	0.27	0.67
PB2 (%DM)	r	0.12	0.79	-0.40	0.15	0.25	0.17	0.56	0.57	-0.13
	P-value	0.76	0.01	0.29	0.71	0.52	0.66	0.12	0.11	0.74
PC (%DM)	r	0.28	-0.04	0.27	0.22	0.18	0.24	-0.58	-0.57	-0.26
	P–value	0.47	0.92	0.48	0.57	0.64	0.53	0.10	0.11	0.49
PA2 (%TP)	r	-0.72	0.11	-0.62	-0.69	0.37	-0.66	0.35	0.29	0.24
	P–value	0.03	0.78	0.08	0.04	0.33	0.05	0.36	0.45	0.54
PB1 (%TP)	r	0.70	-0.23	0.67	0.66	-0.40	0.62	-0.43	-0.38	-0.21
	P–value	0.04	0.55	0.05	0.05	0.28	0.07	0.25	0.32	0.58
PB2 (%TP)	r	0.08	0.80	-0.43	0.12	0.27	0.14	0.58	0.59	-0.12
	P–value	0.83	0.01	0.25	0.77	0.49	0.71	0.10	0.10	0.76

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; PA2: soluble true protein; PB1: insoluble true protein; PB2: fiber-bound protein; PC: indigestible protein.

P = 0.08). The PB2 presented as DM had a positive correlation with Amide II height (r = 0.79, *P* = 0.01).

For apparent nitrogen balance, urinary N excretion tended to have a positive correlation with Amide I area (r = 0.62, *P* = 0.08). Fecal N had a negative amide II area (r = -0.71, *P*= 0.03; Table 4.14). Total N excretion had a negative amide II area (r = -0.79, *P*= 0.01). Milk N tended to have a negative correlation with amide I height (r = -0.67, *P* = 0.05), Amide I to II height ratio (r = -0.60, *P* = 0.09), amide I area (r = -0.63, *P* = 0.07), and total amide area (r = -0.64, *P* = 0.06). Apparent N balance tended to have a positive amide I height (r = 0.60, *P*= 0.09).

4.4.11. Multiple regression analysis for predicting nitrogen utilization using amide spectral *profiles*

Multiple regressions analysis was used to predict the protein profiles and protein subfractions using the Amide spectral features of dietary treatments. The regression analysis showed that the CP could be predicted from Amide I to II height ratio, taking 47% of the total variance. The SCP could be predicted from Amide I height, taking 51% of the total variance. For the predicted energy, tdCP could be predicted from Amide I to II height ratio, taking 48% of the total variance (Table 4.15).

For protein subfractions, PA2 could be predicted from Amide I height, with 53% of the total variance (Table 4.16). The PB1 presented could be predicted from amide I to II height ratio and amide I height, with 48% of the total variance. For the apparent nitrogen balance, fecal excretion could be predicted from α–helix to *β*–sheet ratio, with 47% of the total variance (Table 4.17). Milk N secretion could be predicted from α–helix to *β*–sheet ratio, with 50% of the total variance. Milk N intake could be estimated from amide I height, with 49% of total variance.

Table 4.14. Correlation between the apparent nitrogen balance for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)* and protein molecular structure.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
N intake (g/d)	r	0.31	- 0.14	0.29	0.27	-0.31	0.24	- 0.37	- 0.39	0.19
	P–value	0.41	0.71	0.44	0.49	0.42	0.54	0.32	0.30	0.63
Urinary N	r	0.59	- 0.21	0.52	0.62	- 0.25	0.60	- 0.26	- 0.21	- 0.22
Excretion (g/d)	*P*–value	0.10	0.58	0.15	0.08	0.52	0.09	0.50	0.59	0.57
Fecal N (g/d)	r	- 0.38	- 0.38	- 0.03	- 0.24	- 0.71	- 0.32	- 0.01	- 0.11	0.69
	P–value	0.32	0.32	0.93	0.53	0.03	0.41	0.98	0.79	0.04
Total N	r	- 0.02	- 0.46	0.25	0.12	- 0.79	0.04	- 0.15	- 0.21	0.50
excretion (g/d)	*P*–value	0.96	0.21	0.51	0.76	0.01	0.92	0.70	0.59	0.17
Milk N		- 0.67	- 0.05	- 0.60	- 0.63	- 0.03	- 0.64	0.54	0.49	0.70
(g/d)	*P*–value	0.05	0.91	0.09	0.07	0.93	0.06	0.13	0.18	0.03
Apparent N	r	0.60	0.08	0.44	0.49	0.06	0.50	- 0.53	- 0.49	- 0.36
balance (g/d)	*P*–value	0.09	0.84	0.24	0.19	0.89	0.18	0.15	0.18	0.34
Productive N	r	0.36	0.07	0.20	0.24	0.05	0.25	- 0.35	- 0.34	- 0.04
(g/d)	*P*–value	0.34	0.85	0.60	0.53	0.90	0.52	0.36	0.38	0.91

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate.

Table 4.15. Multiple regression analysis to choose the most important protein spectral parameters to predict basic protein profile and predicted energy profile

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	*P*-value
Basic Protein Profile					
CP (%DM)	Amide I, II ratio	Y = 14.22 + 0.69× Amide I, II ratio	0.47	0.664	0.04
SCP (%CP)	Amide I height, α, β– ratio	Y = 106.08 – 230.78 × Amide I height – 37.00 α, β– ratio	0.79	4.044	<0.01
SCP (%DM)	Amide I height	Y = 8.25 – 14.51 × Amide I height	0.51	0.534	0.03
Predicted energy values					
tdCP (%DM)	Amide I, II ratio	Y = 13.10 + 0.61 × Amide I, II ratio	0.48	0.591	0.04

RSD: residual standard deviation; CP: crude protein; SCP: soluble crude protein; tdCP: truly digestible crude protein.

Table 4.16. Multiple regression analysis to choose the most important protein spectral parameters to predict protein subfractions, ruminal degradable and undegradable subfractions of protein

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: Y= a + b1 × x1 + b2 × x2 +…….	Model R^2	RSD	*P*-value
Protein subfractions					
PA2 (%DM)	Amide I height	Y = 8.25 - 14.51× Amide I height	0.53	0.534	0.03
PB1 (%DM)	Amide I, II ratio	Y = 5.59 + 1.10 × Amide I, II ratio	0.48	1.056	0.04
PA2 (%TP)	Amide I height, α, β– ratio	Y = 117.28 – 253.51 × Amide I height – 42.30 α, β– ratio	0.77	4.399	0.01
PB1 (%TP)	Amide I height	Y = 38.12 + 136.78× Amide I height	0.48	5.036	0.04

RSD: residual standard deviation; PA2: rapidly degradable true protein; PB1: moderately degradable true protein.

Table 4.17. Multiple regression analysis to choose the most important protein spectral parameters to the apparent nitrogen balance

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	*P*-value
Fecal Excretion					
N, g/d	α, β– ratio	Y = –80.20 + 285.92 × α, β– ratio	0.47	37.011	0.04
Milk N					
g/d	α, β– ratio	Y = -76.13 + 366.72 × α, β– ratio	0.50	47.469	0.03
% of N intake	Amide I height	Y = 73.23 - 243.86× Amide I height	0.49	8.978	0.04

RSD: residual standard deviation.

4.5. Discussion

In recent years, intensive dairy and feedlot operations have faced pressure due to the demands of meat and milk production. Improving nitrogen utilization in this system would improve the efficiency of cows, and thus decreases the cost of feed meanwhile increasing the productivity of cows. The protein supplement is the most cost-effective feed source in lactating rations. High producing dairy cows require 14-17% CP in their daily rations (NRC, 2001). The absorbed RUP and microbial protein synthesis are the most important contributors for milk protein production in dairy cows. Carinata meal is a new feed that has not been fully-registered by the Canadian Food Inspection Agency (CFIA) for use in dairy cows' diets. Carinata meal is a co-product from bio-fuel processing of carinata seed (Agrisoma Boisciences Inc. 2015). Carinata crop is characterized by its high yield in semi-arid regions (Agrisoma Boisciences Inc. 2015). Therefore, carinata meal would have a high potential to be a good source of protein to dairy cows in western Canada and the north of the US. To our knowledge, so far only one study investigated the effects of feeding carinata meal as dietary CP supplement for growing dairy heifers (Rodriguez-Hernandez and Anderson, 2018).

The dietary ingredients and chemical compositions of experimental diets fed to lactating dairy cows are presented in Table 4.1. The pelleted products BPPCR and BPPCN were used based on the screening study done by Guevara et al. (2018), who found theses blend-pelleted co-products had the highest feed milk value (FMV) with low glucosinolates levels. The same authors also found adding lignosulfonate to BPP based on canola increased the aRUP and FMV in dairy cows. Thus, we used these results to develop a dairy trial to assess the actual feeding values of these BPPs in high producing dairy cows. The control diet was a commercial diet that is commonly used by dairy producers in western Canada. This diet has a blend of soybean meal, canola meal, and

peas. Peas are typically utilized for improving the durability index of pellets. All diets were formulated to be isonitrogenous and isocaloric. All diets exhibited similar CP and CHO profiles, however, the diet based on canola meal BPPCN and control had a higher indigestible fiber as expressed as ADL compared with diets based BPPCR. These findings are in line with Guevara-Oquendo et al. (2018) who found that the BPP based on carinata meal had lower ADL (%NDF) compared with canola meal (10 vs. 30% NDF). The indigestible fiber in the feed is the main limiting factor for DMI. High producing dairy cows' diets are characterized by high NDF content (30-35% on DM basis). Increasing the ADL content in the diets would increase the gut fill, and hence decrease the energy intake (Dado and Allen, 1995). Adding BPP based on carinata meal would, in turn, increase energy intake, particularly during early lactation stage when the feed intake is limited.

Evaluating the diet using the CNCPS model showed that the BPP based on canola meal tended to have a higher content of soluble true protein fraction (PA2 %TP). This fraction has high Kd values (25 %/h) and less mean retention time in the rumen (4h). The PA2 is used mainly as the primary N source for microbial growth in the rumen (Higgs et al., 2015). The previous study by Guevara-Oquendo (2018) found the BPP2 was higher in Canola BPP compared with BPP based on carinata meal.

The energy values for the different diets were estimated using the NRC dairy model (2001). All diets exhibited the same energy values. Ban et al. (2017) found that the carinata meal had higher TDN and energy values than canola meal, due to the higher protein content (48 vs. 38 %DM). Guevara-Oquendo et al. (2018) reported that the BPP based on canola had a lower TDN and energy values than BPP based on carinata (71 vs 76.5) meal because of the high protein

content. The similar energy values for all diets in the current study is attributed to that all diets has been balanced in N and energy.

There was no significant effect of the dietary treatments on nutrient digestibility of lactating dairy cows. Furthermore, there was no significant effect of BPP on DM, CP and ADF intakes. However, the cows fed the control diet tended to have a higher starch intake, while the cows fed BPPCR tended to have a low NDF intake. The lower NDF intake or high starch intake would decrease the gut fill in the rumen and could increase energy intake in dairy cows (Dado and Allen, 1995). The effect of feeding canola meal on nutrients intake and digestibility have been reported in many studies. However, there is no report on the effect of feeding carinata meal on nutrients digestibility of lactating dairy cows. Rodriguez-Hernandez and Anderson (2018) studied the effect of feeding carinata meal and DDGS on the growth performance of growing dairy heifers. They did not detect any significant impact of carinata meal on DMI compared to DDGS based diet. Guidotti (2018) studied the effects of carinata meal relative to canola meal when fed alone or in combination with wheat DDGS on nutrient intakes and nutrients digestibility in growing beef heifers. They did not detect any effect of the different diets on nutrient intake and digestibility. Using the in situ and in vitro techniques to predict the ruminal and intestinal digestibility of carinata and canola meals, Xin and Yu (2013) reported higher OM digestibility (86 vs. 80%) and CP digestibility (93 vs. 89%) for carinata meal relative to canola meal. Rodriguez-Hernandez (2018) found that dairy heifers fed a diet with solvent-extracted carinata meal at 10% (DM basis) had similar total-tract digestibility of nutrients, with heifers fed the canola meal.

The effect of feeding different blend products based on carinata meal or canola meal on milk production and milk composition has been examined in the current study. To our knowledge, there was no study before on the effect of feeding of carinata meal on milk production or milk

component yield in high producing dairy cows. Dairy cows require an adequate supply of MP supply and amino acids, i.e., lysine and methionine for maintaining high milk yield. In a previous study, Guevara-Oquendo et al. (2018) reported that BPP based on carinata had a high FMV compared to canola meal. The higher feeding value of carinata meal was due to the higher protein content in the carinata meal compared with canola meal. The same authors reported a higher methionine (1.94 vs. 1.76 %DM) and lysine in canola meal (5.90 vs. 4.24 %CP) compared with canola meal. The lysine to methionine ratio was higher in BPP based on canola meal compared with carinata meal (3.0 vs. 2.4). Lysine and methionine are the most limiting factors for milk protein production. The diets in the present study were formulated to have the same protein content for better understanding the MP supply of both diets on milk production. Guidotti (2018) found that feeding diet based on carinata meal alone or in combination with wheat DDGS did not improve the growth performance of growing beef cows relative to canola meal. Rodriguez-Hernandez and Anderson (2018) also did not observe any significant effect of feeding carinata meal on growth performance of growing dairy heifers. To my knowledge, this is the first study demonstrating that carinata meal can be fed and exhibit a similar production performance compared with commonly used feedstuffs in the dairy industry. However, it is warranted to conduct more studies to examine the effect of this new feed using other feeding additives and to compare it with other protein sources such as DDGS or soybean meal either alone or in combination with other protein feeds.

Adding lignosulfonate to blend-pelleted products did not affect protein utilization and hence the milk protein and milk yield. These findings are not in line with a previous study by Wright et al. (2005) who found that adding lignosulfonate improved the N utilization in dairy cows and increased milk yield. The higher N-utilization was attributed to increasing RUP and decrease urinary N excretion in dairy cows. However, these authors formulated a diet with high CP content

compared with our study (17% vs.16%). Thus, further studies are required to examine the effect of adding lignosulfonate to carinata meal in the diet with high CP content. Adding lignosulfonate to BPPCR and BPPCN diet did not influence the apparent digestibility of nutrients. These findings are not in agreement with Wright et al. (2005), who found the ruminal degradable protein (71% vs. 30%) and CP digestibility (74% vs. 71%) were lower for cows fed the canola treated with lignosulfonate compared with the untreated canola. The lack effect of adding lignosulfonate to canola meal or carinata meal was attributed to the lack effect of this feed additive on the ruminal degradable protein.

Income over feed cost (IOFC) is defined as the part of the revenue from milk sold that remains after paying for purchased and farm-raised feed used to produce the milk. IOFC was increased by about $1.07 per cow/d in BPPCR as compared to feeding BPPCN diet. Feeding the cows on BPPCR increased IOFC and dairy herd profitability. Although there was no significant difference among treatments, with using more animals, it would decrease the standard error of the mean, and thus clarify this difference.

In recent years, several studies have been reported that the chemometric methods based on mid-infrared region (MIR) can accurately predict rumen degradability of feed nutrients (Andrés et al., 2005; Ohlsson et al., 2007). Nevertheless, to our knowledge, there is no report on the capability of FTIR spectrometry to predict the N utilization value of different diets with blend-pelleted products based on different protein feeds in high producing dairy cows. Furthermore, there is no study on the association between the spectral features related to the amide region and the production performance of dairy cows. The MIR spectroscopic methods can provide more benefit than NIR analysis because the light absorptivity is greater in the MIR than in the NIR range. Moreover, the MIR region (ca. 4000-400 cm^{-1}) contains information on the fundamental molecular

vibrations, which are stronger than the overtone absorptions observed in NIR (4000-12500 cm^{-1}; Manley, 2014). As a result, generally MIR has higher sensitivity and precision than NIR and therefore could give a better insight into the chemical composition of the feed sample. Yu (2005) reported that protein secondary structures are highly correlated with digestive behavior and protein makeup. The amide structures have been reported to be affected by ruminal and intestinal digestibility of protein through changing the solubility of protein and the access of proteolytic enzymes to protein molecules in the gastrointestinal tract of dairy cattle (Theodoridou and Yu, 2013a). In the current study, there was no significant effect of different diets on the secondary structure of amide region, indicating a similar protein utilization potential for the various treatments in dairy cows.

The correlation between the amide structures would explore the association between the inherent molecular structures of feed with predictions of production performance in dairy cows. The results in the current study showed that fecal nitrogen exertion was significantly correlated with the amide II region, while the milk nitrogen was found to be significantly associated with amide I region and the secondary structure of amide I region. These correlations in data suggests that protein molecular structure is strongly affects N-utilization in dairy cows. The differences in the amide I and amide II spectral intensities demonstrate the quantitative differences in protein contents and indigestible protein content, respectively (Peng et al., 2014). The Amide I to II and α-helix to β-sheet ratios reflect the N utilization in dairy cows (Yu, 2007).

4.6. Conclusions

The blend-pelleted products based on carinata meal as a new co-product from bio-fuel processing industry was equal to the other pelleted products based on canola meal as a protein source for dairy cattle without affecting the lactational performance of dairy cows. Adding

lignosulfonate to BPP did not improve the protein utilization in dairy cows. Further research is warranted to determine the influence of adding other feed additives on carinata meal degradability and to compare the lactational performance of cows fed carinata meal to those fed more traditional protein supplements such as soybean meal. The molecular spectroscopy could detect inherent structural characteristics in the blend-pelleted products based on different bio-energy co-products. The molecular structural features related to the protein region were highly associated with the protein utilization in dairy cows.

5. ASSOCIATION BETWEEN MOLECULAR STRUCTURE FEATURES AND METABOLIC CHARACTERISTICS OF BLEND-PELLETED PRODUCTS BASED ON BIOENERGY CO-PRODUCTS IN HIGH PRODUCING DAIRY COWS

5.1. Abstract

The main objectives of this study were to detect the impact of feeding newly developed blend-pelleted products based on carinata meal (BPPCR) or canola meal (BPPCN) in combination with pulse screenings and lignosulfonate on ruminal fermentation characteristics, ruminal degradability and intestinal digestion in high producing dairy cows, and also to examine the changes of amide molecular structure spectral profiles in relation to ruminal degradability and intestinal digestion of BPPCR diet and BPPCN diet in dairy cows. Three mid-lactating cannulated Holstein cows were randomly assigned to one of the following three dietary treatments: Control = control diet (common barley-based diet in western Canada); BPPCR = basal diet supplemented with 12.3 %DM BPPCR (carinata meal 71.4 % + pea screenings 23.8 % + lignosulfonate 4.8 %DM), and BPPCN = basal diet supplemented with 13.3 %DM BPPCN (canola meal 71.4 % + pea screenings 23.8 % + lignosulfonate 4.8 %DM) in a 3×3 Latin square design. Each experimental period lasted for 21 days with 14 days for adaptation and seven days of sampling. The results showed that there were significant differences ($P < 0.05$) among diets in pH duration and rumen pH area, where the BPPCN diet exhibited the highest pH duration and pH. The control diet was higher ($P < 0.05$) in total VFA rumen concentration (138 mmol/L) relative to BPPCN. There was no dietary effect ($P > 0.10$) on the concentration of rumen ammonia. There was no effect ($P > 0.10$) of dietary treatments on in situ ruminal degradation kinetics of dietary nutrients. There was no significant difference ($P > 0.10$) among the different diets on intestinal digestion of nutrients. The predicted metabolizable protein was not affected ($P > 0.10$) by different dietary treatments. Similarly, the

feed milk values were not affected ($P > 0.10$) by different diets. The blend-pelleted products exhibited significant correlation with ruminal and intestinal digestion of amide region. In conclusion, the blend-pelleted products based on carinata meal as a new co-product from bio-fuel processing industry was equal to the other pelleted products based on canola meal as a protein source for dairy cattle without affecting the ruminal fermentation features. The molecular spectroscopy could identify structural characteristics in dietary treatments based on the two co-products.

5.2. Introduction

The use of biofuel industry by-products as feedstuffs for dairy cows is a realistic option to decrease feed cost and increase the production efficiency of high producing dairy cows (Canola Council of Canada, 2015). New co-product from bio-fuel processing is the carinata meal. Carinata meal is a good source of crude protein (CP) about 48% CP (Xin and Yu, 2013a). However, carinata meal is characterized by its higher level of rumen degradable protein compared with canola meal (Ban et al., 2017). In recent years, Canada has become the second country in terms of pea production (Hickling et al., 2003). In 2014, Saskatchewan grew about 64% of the dry pea crop and 90% of chickpea crop of the total Canadian pea production (Saskatchewan Pulse Growers, 2015). Pea is a high in protein, at about 24% dry matter (DM) and also contain a high level of starch 46% DM (Hickling et al., 2003). The rumen degradable protein (RDP) of peas is about 78% (Kudlinskiene et al., 2016). Decreasing the rumen degradability of protein supplements is an essential strategy used to improve the dietary amino acids (AAs) supply to the small intestine. This concept assumes enhancing milk production from increased amino acids supply to the lactating dairy cow.

Because of the high level of RDP in canola meal, carinata meal, and pea screenings, it is essential to slow down the degradation (extent and rate) of ruminal degradation (Schwab, 1995). The most common methods to maximize the utilization of protein and protected the AAs are heat treatments and feed additives. Heat treatments include techniques such as pelleting, steam flaking, dry roasting, etc. (Jansen, 1991; Riaz, 2007). Heat treatments are vital to improving the nutritional, chemical, physical, hygienic, and other animal feed characteristics (Lević et al., 2010). Feed additives such as formaldehyde (Crooker et al., 1983), tannins (Chung et al., 2013), lignosulfonate (LSO_3), and xylose (McAllister et al., 1993) could decrease RDP of protein in the different ration.

There are many methods for feed evaluation such as wet chemistry analysis; however, this technique could damage the main structure of samples (Yu et al., 2014). It has been reported by Yu (2005) that feeding value is influenced by the inherent molecular structure of amide region. The FTIR spectroscopy with attenuated total reflectance is a direct, rapid, non-invasive, and non-destructive bioanalytical technique used to examine the infrared spectrum of absorptions or emissions of liquid, gas, or solids (Smith, 2011). FTIR has been used to detect the molecular structure for different crop varieties, feed ingredients, and to study the effects of feed processing on protein- and carbohydrate-related structures (Abeysekara et al., 2013; Peng et al., 2014; Xin and Yu, 2013a,b). To our knowledge, there has been no study to detect how the BPP based on different co-products from bio-oil or bio-fuel processing (i.e., carinata or canola meal) could induce protein molecular structures changes, or how these changes could influence the ruminal degradation and intestinal digestibility in high producing dairy cows.

The main objectives of this study were: (1) to detect the effects of feeding newly developed blend-pelleted products based on new protein feed of carinata meal or canola meal in combination with pea screenings and lignosulfonate on ruminal degradation and intestinal digestion in dairy

cows; and (2) to assess the correlation between molecular structure features related to amide region and ruminal degradation and intestinal digestion in high producing dairy cows.

5.3. Materials and Methods

5.3.1. Animals and experimental design and diets

Three multiparous lactating Holstein cows were used in a 3×3 Latin square design with three different dietary treatments. Each experimental period lasted for 21 days, consisting of 14 days of diet adaptation and seven days of sample collection. The three cows were housed in individual tie-stalls at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, Canada). The cows were randomly assigned to one of the following three diets: Control = control diet: common barley-based diet in western Canada (6.2 % canola meal + 2.2 % soybean meal + 3.9 % peas), BPPCR diet: basal diet supplemented with 12.3%DM BPPCR (carinata meal 71.4 % + pea screenings 23.8 % + lignosulfonate 4.8 %DM), and BPPCN diet = basal diet supplemented with 13.3 %DM BPPCN (canola meal 71.4 % + pea screenings 23.8 % + lignosulfonate 4.8 %DM).

5.3.2. Ruminal pH measurements

Rumen pH was measured every 5 minutes in the last three days for each experimental period using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006). The pH probes were standardized by using two buffers (pH 7 and 4). All probes were collected from each cow in the last third day of pH measurement, washing them, and downloading the data. The electrode signal in mV was converted to pH data using the calibration slope. The pH probes detected the slope by measuring the difference in the mV reading of two different buffers (pH 7 and 4) and divided it by the difference in pH of the buffers.

5.3.3. Rumen fluid collection

In the last day of each experimental period (starting on day 21 at 0800 h), the ruminal fluid was collected over 24 hours every 3 hours (0, 3, 6, 9, 12, 15, 18, 21, 24 h). About 250-mL of ruminal liquid were collected from four different locations of the rumen (ventral, anterior, posterior, and rumen mat). After that, the ruminal fluid went through two layers of cheesecloth and solids discarded. Two 10-mL of the filtrate samples were sub-sampled into 15-mL centrifuge tubes (Fisher Scientific, Waltham, MA). One of these samples was added to a tube containing 2-mL of 25% metaphosphoric acid for VFA analysis and the other one of these samples attached to a tube containing 2-mL of 1% sulphuric acid for ammonia analysis. All samples were stored at –20 °C.

The frozen ruminal volatile fatty acid (VFA) samples were melted overnight at 4°C. Then the samples were thoroughly mixed and centrifuged at 12,000 g for 10 min at 4°C using a Beckman Centrifuge (Model Avanti J-E; Palo Alto, CA, USA). About 1.0-mL of this sample was placed into microcentrifuge tubes (VWR TM 1.5 mL Microcentrifuge tube with snap cap, Radnor, PA, USA). After that, samples were centrifuged at 16,000 g for 10 min at 4°C using a Microcentrifuge (Beckman Coulter TM, Brea, CA, USA). An internal standard containing 300 μL isocaproic acid, 20-mL of 25% metaphosphoric acid, and double distilled water (ddH_2O) were mixed with 1-mL of the supernatant sample in a GC vial (Agilent TechnologiesTM, Santa Clara, CA, USA) to determine the concentration of VFA by comparison of peak areas using an Agilent 6890 series Gas chromatography system (Agilent Technologies TM, Santa Clara, CA, USA) with an Agilent 7683 series 5 μL injector, Zebron ZB-FFAP high performance GC capillary column (30 m × 320 μm × 0.25 μm, Phenomenex, Torrance, CA, USA) and an Agilent split focus liner (Agilent TechnologiesTM, Santa Clara, CA, USA). Samples were prepared daily at 4°C to avoid

volatilization until analysis. To build a calibration curve, acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, and isocaproic acids were used as a mixed standard.

For ammonia analysis, frozen samples were kept overnight at 4°C, vortexed, and centrifuged at 12,000 g for 10 min at 4°C using a Beckman Centrifuge (Model Avanti J-E; Palo Alto, CA, USA). After that, 1.0-mL of the sample was placed in microcentrifuge tubes (VWR TM 1.5-mL Microcentrifuge tube with snap cap, Radnor, PA, USA) and centrifuged at 16,000 g for 10 min at 4°C using a Microcentrifuge (Beckman Coulter TM, Brea, CA, USA). Ammonia concentration of ruminal fluid was analyzed using the phenol-hypochlorite method of Broderick and Kang (1980).

5.3.4. Cornell Net Carbohydrate and Protein System (CNCPS V.6.5)

In the CNCPS (Higgs et al., 2015), protein is divided to PA1 ammonia (Kd =200%/h), PA2 soluble true protein (Kd =10-40 %/h), PB1 (moderately degradable true protein, Kd = 3-20 %/h), PB2 (slowly degradable true protein, Kd = 4-9%/h) and PC (unavailable protein) based on their rumen degradation features. The carbohydrate partition is described by Higgs et al. (2015). The eight subfractions include CA1, CA2, CA3, CA4, CB1, CB2, CB3, and CC, based on rumen fermentation and microbial activity on carbohydrate availability (Van Amburgh et al., 2015). The CA1 fraction is VFA consisting mainly of acetate, propionate, and butyrate, which are not degradable (0 %/h). The CA2 fraction is lactic acid with a degradation rate of 7%/h. The CA3 fraction degrades at 5%/h. The CA4 fraction with Kd 50%/h. The CB1 fraction with Kd rates equal to 30%/h. The CB2 fraction degrades at 30%/h. The CB3 fraction with Kd rates equal to 6%/h. The CC, mostly plant cell walls containing lignin, is considered undegradable. The Kp is 13.75 %/h (mean retention time = 7.3 h) for CA4, PA1 and PA2, and 7.60 %/h for other CB1 and CB2

and PB1 fractions (mean retention time = 13.2 h) and 1.66%/h for PB2 and CB3 (mean retention time = 60.2 h).

5.3.5. Rumen incubation procedure and sample analysis

An in situ method was used to determine rumen degradation kinetics as described by Yu et al., (2003). The in situ procedure included weighing 7 g of each diet in each number-coded nylon bag (10 x 20 cm) with multiple-bags for each treatment and each incubation 0, 3, 6, 9, 12, 24, and 48 h. The pore size of the nylon bag was ca. 41 μm. These bags were tied about 2 cm below the top, allowing a ratio of a sample size to bag surface area of 39 mg/cm^2. The rumen incubations were performed with three cannulated cows according to the ‘‘gradual addition/all-out’’ schedule (the bags were inserted sequentially and retrieved at the same time) and incubated in the rumens for 3, 6, 9, 12, 24, and 48 h). After incubation, the bags were collected from the rumen and washed with cool water by hand for six times with ca. 10 bags each round. The 0 h bags were washed under the same conditions four times. After washing the bags, the bags were dried for analyzing DM at 55 °C for 48 h by placing all bags on stainless steel trays in a forced-air drying oven. All dried bags were moved to lab room conditions (temperature room at 21 °C) for at least 24 h, then bag + string + residue were weighed. The samples were ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England) for chemical analysis. In situ samples were analyzed for ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 990.03), neutral detergent fiber (NDF), and, starch (ST; Hall, 2009).

5.3.6. Measurement of rumen degradation kinetics of feed nutrients using the in situ technique

Degradation characteristics of DM, organic matter (OM), CP, NDF, and ST were determined using the first-order kinetics degradation model described by Ørskov and McDonald

(1979) and modified by Tamminga et al. (1994). The results were estimated using the nonlinear (NLIN) procedure of SAS 9.4 and iterative least-squares regression (Gausse Newton method) as in the following equation:

$$R(t) = U + D \times e^{-Kd \times (t - T0)},$$

where, R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (h^{-1}), and T0 = lag time (h).

The rumen undegradable (R) or bypass (B) values of nutrients on a percentage basis were calculated according to NRC Dairy (2001) as the following equation:

$$\%BDM, BCP \text{ or } BNDF = U + D \times Kp / (Kp + Kd)$$

$$\%BST = 0.1 \times S + D \times Kp / (Kp + Kd),$$

where, Kp stands for estimated passage rate from the rumen (4.5%/h); S stands for a soluble fraction (%). The factor 0.1 in the formula represents the approximate 100 g/kg of the soluble fraction (S) that escapes rumen fermentation (Tamminga et al., 1994).

The rumen undegradable or bypass DM, and starch (ST) in g/kg DM were calculated as the following equation:

$$BDM \text{ or } BST \text{ (g/kg DM)} = DM \text{ or } ST \text{ (g/kg DM)} \times \%\ BDM \text{ or } BST$$

Except for the rumen undegradable protein (RUP) and rumen bypass protein (BCP) were calculated differently in the Dutch model (Tamminga et al., 1994) and NRC Dairy 2001 model (NRC, 2001):

$$BCP^{DVE} \text{ (g/kg DM)} = 1.11 \times CP \text{ (g/kg DM)} \times RUP \text{ (\%)},$$

$$RUP^{NRC} \text{ (g/kg DM)} = CP \text{ (g/kg DM)} \times RUP \text{ (\%)},$$

where, 1.11 is the regression coefficient between in situ RUP and in vivo RUP (Yu et al., 2002; Tamminga et al., 1994).

The effective degradability (ED), or extent of degradation, of each nutrient was predicted according to NRC (2001) as the following equation:

$$\%EDDM\ (EDCP\ or\ EDST) = S + D \times Kd / (Kp + Kd)$$

$$EDDM\ (CP\ or\ ST) = DM\ (CP\ or\ ST)\ (g/kg\ DM) \times \%EDDM\ (EDCP\ or\ EDST)$$

5.3.7. Evaluation of Intestinal Digestibility of Feed Nutrients Using In Vitro Techniques

Intestinal digestion was evaluated using the three-step in vitro procedure described by Calsamiglia and Stern (1995). In vitro processing included the following steps: 1) dried ground residues containing 15 mg of N after 12 h ruminal preincubation were placed into a 50 ml centrifuge tube; 2) a 10 ml of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added, vortexed, and incubated for 1 h at 38 ºC in a water bath, 3) a 0.5 ml of 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P-7545) were added, vortexed and incubated at 38 °C for 24 h vortexing every 8 h approximately; 4) a 3 ml of TCA was added in order to stop enzymatic hydrolysis; 5) the tubes were vortexed and sit samples for 15 min at room temperature; 6) all samples were centrifuged for 15 min at 10000 g and supernatant (5 ml) analyzed for soluble N by the Kjeldahl method. The intestinal digestion of protein was measured according to TCA-soluble N divided by the amount of N in the rumen residue sample (Gargallo et al., 2006; Calsamiglia and Stern, 1995).

5.3.8. Prediction of truly digestible protein in the small intestine using the National Research Council (NRC 2001) Method

The metabolizable protein (MP) is as a composed of three major contributory protein sources using the NRC (2001) model as the following equation:

$$MP\ (g/kg\ DM) = AMCPNRC + ARUPNRC + AECP,$$

where, AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine (Theodoridou and Yu, 2013; NRC, 2001).

The DBP based on data from the NRC-2001 mode reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on the energy available for microbial fermentation in the rumen. Thus, the DPBNRC was calculated as follows:

$$DPBNRC\ (g/kg\ of\ DM) = RDPNRC - 1.18 \times MCPTDN.$$

The FMV was estimated on the characteristics of protein from NRC, 2001 model. The efficiency of use of metabolizable protein for lactation was assumed to be 0.67 (NRC, 2001), and protein composition in milk was considered to be 33 g protein / 1 kg of milk.

5.3.9. Collecting spectra related to the protein primary and secondary structural components

The molecular spectral features related to the amide region were collected using molecular spectroscopy of FTIR (JASCO 4200, JASCO International Co. Ltd., Tokyo, Japan). The spectra were generated in the mid-IR (ca. 4000–800 cm^{-1}) range. The FTIR spectra were performed by using OMNIC 7.3 (Spectra–Tech, Madison, WI, USA).

5.3.10. Statistical analysis

The data were analyzed using Proc Mixed SAS 9.4 (SAS Institute, Cary, NC) to examine digestibility, ruminal fermentation, and ruminal pH profile by using the following model:

$$Y_{ijkl} = \mu + P_j + C_k + T_l + E_{ijkl},$$

where, Y_{ijkl} was the dependent variable, μ was the overall mean, $P_{j(i)}$ was the fixed effect of j^{th} period, $C_{k(i)}$ was the random effect of k^{th} cow, T_l was the fixed effect of l^{th} dietary treatment, and E_{ijkl} was the residual error.

Means effects were detected by using the LSMEANS procedure. Normality was tested using the univariate procedure of SAS software with the Shapiro-Wilk test. Differences were declared significant if $P < 0.05$ and values of $0.05 < P < 0.10$ were interpreted as tendencies towards significance.

The correlation between the functional groups related to protein region (Amide I, II peak highest and areas, α–helix, *β*–sheet and their ratio) and ruminal degradability and intestinal digestibility was analyzed by using the PROC CORR procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Rank correlation with the SPEARMAN option and normality test with the UNIVARIATE option were used in the correlation study.

Multiple regression analysis (with model variable selection method) used to select the best functional groups that would explain ruminal degradability and intestinal digestion by using the PROC REG procedure of SAS by the following model: Y= spectral parameter 1 + spectral parameter 2 + spectral parameter 3 + spectral parameter 4 + ... + error. The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". All variables left in the final prediction models were significant at the 0.05 level. Residual analysis was performed and normality was tested the Univariate procedure of SAS with Normal and Plot options.

5.4. Results

5.4.1. Ruminal pH profile

The mean pH and the maximum pH were not affected ($P > 0.10$) by different dietary treatments (Table 5.1); however, the minimum pH tended ($P = 0.07$) to be lower in BPPCN (5.14) compared with the control and BPPCR diets (average 5.45; Table 5.1). Our results showed that BPPCN diet was significantly ($P > 0.05$) higher in duration of pH < 5.8 (930 min/d) and duration pH < 5.5 (285 min/d) compared with BPPCR diet and control diet.

5.4.2. Ruminal fermentation

The total VFA was increased in the control diet ($P < 0.05$; 138 mmol, L) compared with BPPCR diet and BPPCN diet (averaging 119 mmol/L; Table 5.2). Acetate tended ($0.05 < P < 0.10$) to be higher in control diet (78.8 mmol/L) compared with BPPCR and BPPCN diets (averaging 70.35 mmol/L). Our results showed that the control diet had higher propionate (37.2 mmol/ L) compared with BPPCR diet and BPPCN diet (averaging 29.05 mmol/L). Iso-butyrate, butyrate, iso-valerate, and iso-caproate were not affected ($P > 0.10$) by different dietary treatments.

For ammonia, there was no effect of diets ($P > 0.10$) on ammonia concentration; however, when comparing control diet with the averaging of BPPCR diet and BPPCN diet, the control tended ($0.05 < P < 0.10$) to be higher in ammonia relative to other different dietary treatments.

5.4.3. Ruminal degradation of protein and carbohydrate subfractions

Ruminal degradable protein subfractions such as RDPA2, RDPB1, RDPB2, and TRDP and ruminal undegradable protein subfractions such as RUPA2, RUPB1, and RUPB2 were not affected ($P > 0.10$) by different dietary treatments (Table 5.3). However, BPPCN diet was higher ($P < 0.05$) in TRUP (4.50 %DM) compared with BPPCR diet and BPPCN diet (averaging 4.16 % DM).

Table 5.1 Ruminal pH pattern for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Ruminal pH						
Mean	6.00	6.09	5.74	0.083	0.17	0.10
Minimum	5.51	5.41	5.14	0.053	0.07	0.04
Maximum	6.42	6.69	6.49	0.181	0.63	0.80
Duration, min/d						
pH < 5.8	172.50^{b}	257.50^{b}	930.00^{a}	14.216	<0.01	<0.01
pH < 5.5	12.50^{b}	45.00^{b}	285.00^{a}	21.262	0.02	0.01
Area, pH × min/d						
pH < 5.8	23.80^{c}	41.41^{b}	218.90^{a}	1.255	<0.01	<0.01
pH < 5.5	0.77^{c}	4.39^{b}	42.33^{a}	4.832	0.04	0.02

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; $^{a-c}$ Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method.

Table 5.2 Ruminal fermentation characteristics for high producing dairy cows fed total mixed ration with blend-pelleted products (BPP)[*].

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Total VFA (mmol, L)	138.22[a]	121.79[ab]	117.01[b]	3.531	0.02	0.04
VFA (mmol /L)						
Acetate	78.84	70.67	70.03	2.321	0.08	0.22
Propionate	37.19[a]	30.40[b]	27.70[b]	0.983	<0.01	<0.01
Iso-butyrate	0.90	0.72	0.73	0.067	0.19	0.36
Butyrate	16.87	15.50	14.55	0.811	0.24	0.13
Iso-valerate	1.29	1.09	1.13	0.111	0.45	0.65
Valerate	2.60[a]	2.11[b]	2.08[b]	0.087	0.02	0.15
Iso-caproate	0.71	0.65	0.77	0.088	0.66	0.43
Caproate	2.16[b]	2.43[a]	2.69[a]	0.071	<0.01	<0.01
NH_3-N (mg/dL)	5.60	5.01	4.19	0.469	0.12	0.06

[*]Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; VFA: volatile fatty acids.

Similarity, ruminally degradable carbohydrate subfractions such as RDCA4, RDCB1, RDCB2, RDCB3, and TRDC and ruminal undegradable carbohydrate subfractions such as RUCA4, RUCB1, RUCB2, RUCB3, and TRUC were not affected ($P > 0.10$) by different dietary treatments. However, when comparing control diet with BPPCR diet and BPPCN diet, TRDC tended to be higher in BPPCR diet (46.3 % DM) compared with control diet and BPPCN diet (averaging 45.3 % DM).

5.4.4. In situ rumen degradation kinetics of chemical profiles

Table 5.4 shows in situ ruminal degradation kinetics of dry matter. The rate of degradation (Kd) was significantly ($P < 0.05$) higher in the control diet (9.1 %/h) compared with BPPCR diet and BPPCN diet (averaging 7.2 1 %/h). However, the other ruminal degradation parameters of DM such as degradable fractions (D), undegradable fractions (U), rumen bypass dry matter (BDM) when expressed as a percentage of DM or g/kg of DM, and effectively degraded dry matter (EDDM) when expressed as a percentage of DM or g/kg of DM were unaffected ($P > 0.10$) by dietary treatments. Dietary treatments did not affect ($P > 0.10$) ruminal degradation of organic matter (OM), starch (ST), and crude protein (CP; Tables 5.5, 5.6, and 5.7). In situ ruminal degradation of OM, ST, and CP such as Kd, D, U, rumen bypass organic matter (BOM) when expressed as a percentage of OM or g/kg of OM, effectively degraded organic matter (EDOM) when expressed as a percentage of OM or g/kg of OM, rumen bypass starch (BST) when expressed as a percentage of ST or g/kg of ST, effectively degraded starch (EDST) when expressed as a percentage of ST or g/kg of ST, rumen bypass crude protein (BCP) in DVE/OEB system when expressed as a percentage of CP or g/kg of CP, effective degraded crude protein (EDCP) when expressed as a percentage of CP or g/kg of CP were not affected ($P > 0.10$) by dietary treatments.

Table 5.3 Ruminal degradable and undegradable subfractions of protein and carbohydrates for the total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Ruminal degradable protein fractions (%DM)						
RDPA2	4.92	4.96	4.69	0.051	0.24	0.17
RDPB1	5.70	5.53	6.35	0.070	0.11	0.08
RDPB2	0.07	0.06	0.03	0.038	0.82	0.61
TRDP	10.69	10.55	11.07	0.089	0.23	0.16
Ruminal undegradable protein fraction (%DM)						
RUPA2	0.66	0.67	0.63	0.006	0.24	0.16
RUPB1	2.28	2.21	2.54	0.026	0.10	0.07
RUPB2	0.20	0.19	0.10	0.115	0.84	0.64
TRUP	4.16[b]	4.15[b]	4.50[a]	0.013	0.05	0.03
Ruminal degradable carbohydrate fraction (%DM)						
RDCA4	4.80	5.45	5.90	0.447	0.53	0.41
RDCB1	26.70	23.95	25.30	0.574	0.27	0.98
RDCB2	11.98	14.01	12.57	0.798	0.44	0.75
RDCB3	25.29	26.18	24.37	0.447	0.31	0.26
TRDC	46.19	46.25	44.36	0.756	0.21	0.09

Table 5.3 *Cont'd.* Ruminal degradable and undegradable subfractions of protein and carbohydrates for the total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Ruminal undegradable carbohydrate fraction (%DM)						
RUCA4	5.93	5.32	5.62	0.128	0.27	0.98
RUCB1	1.94	2.27	2.04	0.128	0.44	0.76
RUCB2	42.15	43.64	40.62	0.747	0.31	0.26
RUCB3	4.01	3.62	3.83	0.064	0.21	0.89
RUCC	56.17	57.27	54.73	0.485	0.25	0.20
TRUCC	2.13	2.43	2.62	0.198	0.53	0.41

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP; RUPA2: ruminally escaped PA2; RUPB1: ruminally escaped PB1; RUPB2: ruminally escaped PB2; RUPC: ruminally escaped PC; TRUP: total ruminally escaped CP; RDCA4: ruminally degraded CA4; RDCB1: ruminally degraded CB1; RDCB2: ruminally degraded CB2; RDCB3: ruminally degraded CB3; TRDC: total ruminally degraded CHO; RUCA4: ruminally escaped CA4; RUCB2: ruminally escaped CB2; RUCB3: ruminally escaped CB3; RUCC: ruminally escaped CC; TRUCC: ruminally escaped CHO

Table 5.4 In situ rumen degradation kinetics of dry matter (DM) with total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*- value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen DM degradation						
Kd (%/h)	9.07[a]	7.84[ab]	6.49[b]	0.444	0.04	0.02
T0 (h)	0.27	0.00	0.27	0.156	0.44	0.53
S (%)	27.68	28.26	27.99	0.775	0.88	0.99
D (%)	49.26	47.88	50.25	1.983	0.72	0.53
U (%)	23.05	23.86	21.76	1.919	0.75	0.51
BDM (=RUDM, g/kg DM)	394.21	416.60	421.93	13.964	0.30	0.29
EDDM (=RDDM, g/kg DM)	605.79	583.40	578.07	13.964	0.30	0.29
BDM (=%RUDM)	39.42	41.66	42.19	1.397	0.30	0.29
EDDM (=%RDDM)	60.58	58.34	57.81	1.397	0.30	0.29

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; DM: Dry Matter; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable degradable fractions; BDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

Table 5.5. In situ rumen degradation kinetics of organic matter (OM) of the total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*- value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen OM degradation						
Kd (%/h)	10.30	6.45	8.42	0.764	0.13	0.97
T0 (h)	0.27	0.00	0.77	0.390	0.44	0.26
S (%)	27.68	28.26	27.99	0.775	0.88	0.99
D (%)	49.26	47.88	51.70	1.387	0.26	0.14
U (%)	23.05	23.86	20.31	1.404	0.28	0.14
EDOM (g/kg DM)	569.52	548.14	566.91	15.265	0.60	0.69
BOM (g/kg DM)	371.31	387.02	370.55	16.560	0.75	0.69
%EDOM	60.54	58.61	60.49	1.711	0.69	0.69
%BOM	39.46	41.39	39.51	1.711	0.69	0.69

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; OM: Organic Matter; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable fractions; BOM: rumen bypass organic matter; EDOM: effective degradability of organic matter.

Table 5.6. In situ rumen degradation kinetics of starch (ST) of total mixed ration with blend-pelleted products (BPP)* lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen Starch degradation						
Kd (%/h)	26.27	31.09	21.88	8.057	0.74	0.53
T0 (h)	1.09	0.86	1.14	0.795	0.96	0.89
S (%)	10.86	4.34	8.58	2.861	0.36	0.94
D (%)	87.82	91.59	90.22	1.994	0.47	0.88
U (%)	0	3.96	2.81	2.026	0.53	0.81
BST (g/kg DM)	38.71	32.43	41.36	8.443	0.83	0.69
EDST (g/kg DM)	215.96	195.08	196.10	18.083	0.24	0.36
%BST	15.22	14.36	17.10	3.304	0.89	0.68
%EDST	86.61	82.33	84.38	2.107	0.51	0.98

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; St: Starch; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable fractions; BST: rumen bypass or undegraded feed starch; EDST: effective degraded starch.

Table 5.7. In situ rumen degradation kinetics of crude protein (CP) of the total mixed ration with blend-pelleted products (BPP)* lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
In situ rumen CP degradation						
Kd (%/h)	5.79	8.64	5.66	1.387	0.33	0.41
T0 (h)	0.86	2.43	3.34	1.409	0.51	0.38
S (%)	27.13	26.70	26.51	2.856	0.99	0.91
D (%)	60.17	57.75	62.15	2.718	0.57	0.39
U (%)	12.99	15.42	11.17	5.904	0.90	0.74
%BCP=%RUP	39.01	36.64	39.39	3.437	0.84	0.73
RUP (g/kg DM, NRC)	56.61	49.20	47.92	3.477	0.39	0.39
BCP (g/kg DM, DVE)	62.83	54.62	53.19	3.862	0.39	0.39
%EDCP (=%RDP)	60.99	63.36	60.61	3.437	0.84	0.73
EDCP (=RDP, g/kg DM)	82.85	87.05	80.74	8.177	0.86	0.70

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; CP: crude protein; Kd: the rate of degradation of D fraction (%/h); T0: lag time; S: washable fraction; D: degradable fractions; U: undegradable degradable fractions; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein.

5.4.5. Intestinal digestion of feed nutrients

For intestinal digestion of DM (Table 5.8), BPPCN diet was tended ($0.05 < P < 0.10$) to be higher in intestinal digestibility of rumen bypassed DM (dBDM; 47.80 %BDM) compared with control diet and BPPCR diet (averaging 41.05 %BDM). The intestinal digestible rumen bypassed DM (IDBDM) when expressed as a percentage of BDM, and g/kg of BDM tended to be affected ($0.05 < P < 0.10$) by different diets. Total digestible of DM (TDDM) when expressed as a percentage of DM was not affected ($P > 0.10$) by different diets. There was no dietary effect ($P > 0.10$) on intestinal digestion of CP such as digestible intestinal protein (IDP) when expressed as a percentage of CP and g/kg of DM and total digestible protein (TDP) when expressed as a percentage of CP and g/kg of DM by different diets. Furthermore, there was no dietary effect ($P > 0.10$) on intestinal digestion of ST such as intestinal digestible starch (IDBST) when expressed as a percentage of BST and g/kg of DM and total digestible starch (TDST) when expressed as a percentage of DM and g/kg of DM by different diets.

5.4.6. Truly absorbed metabolizable protein

Table 5.9 shows predicted truly absorbed metabolizable protein in which there was no significant difference ($P > 0.10$) among diets in rumen- synthesized microbial protein truly absorbable, rumen-undegradable protein truly absorbable, and endogenous rumen protein truly digested in the small intestine for the different dietary treatments. There was no dietary effect ($P > 0.10$) on total metabolizable protein (MP) in the small intestine. Moreover, degraded protein balance (DPB) and feed milk value (FMV) were not affected ($P > 0.10$) by different diets.

Table 5.8 Intestinal digestion and availability of total mixed ration with blend-pelleted products (BPP)* in lactating dairy cows.

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
DM intestinal digestion						
dBDM (% BDM)	39.57	42.53	47.80	1.956	0.09	0.05
IDBDM (% BDM)	15.60	17.57	20.27	1.002	0.07	0.04
IDBDM (g/kg DM)	61.70	72.97	86.03	6.458	0.13	0.08
TDDM (%DM)	75.93	76.26	77.94	1.130	0.21	0.12
CP intestinal digestion						
dIDP (%)	76.33	77.03	70.27	3.264	0.53	0.32
IDP (% CP)	29.83	28.00	27.70	2.520	0.82	0.71
IDP (g/kg DM)	40.17	37.10	36.83	2.019	0.50	0.51
TDP (%CP)	89.35	91.48	89.71	1.341	0.62	0.73
TDP (g/kg DM)	123.03	124.13	117.57	5.352	0.33	0.18
Starch intestinal digestion						
dBST (%BST)	94.03	94.13	94.13	2.856	1.00	0.99
IDBST (%BST)	14.47	13.50	16.13	3.419	0.91	0.71
IDBST (g/kg DM)	6.17	4.40	7.57	2.419	0.78	0.59
TDST (%DM)	99.27	99.13	99.03	0.292	0.86	0.67
TDST (g/kg DM)	219.96	201.12	204.23	17.619	0.19	0.41

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; dBDM: intestinal digestibility of rumen bypassed DM, calculated as:(BDM-DM residual after 48h rumen incubation) / BDM × 100; IDBDM: intestinal digestible rumen bypassed DM, calculated as: BDM × dBDM; TDDM: total digestible DM, calculated as: EDDM + IDBDM; IDP: intestinal digestible protein, calculated as: BCP × dIDP; dIDP: intestinal digestibility of rumen undegraded protein; TDP: total digestible protein, calculated as: EDCP +IDP; dBST: intestinal digestibility of rumen bypassed ST, calculated as:(BST-ST residual after 48h rumen incubation) / BST × 100; IDBST: intestinal digestible rumen bypassed ST, calculated as: BST × dBST; TDST: total digestible ST, calculated as: EDST + IDBST.

5.4.7. Correlation analysis between amide spectral features and ruminal degradation and intestinal digestion

For ruminal degradability of protein subfractions, RDPA2 had a negative correlation with amide I height (r = -0.72, *P*= 0.03) and amide I area (r = 0.69, *P*= 0.04; table 5.10). The RDPB1 had a positive correlation with amide I height (r = 0.68, *P*= 0.04) and tended to have a positive correlation with amide I height (r = 0.68, *P*= 0.04), amide I area (r = 0.64, *P*= 0.06), and total amide area (r = 0.60, *P*= 0.09). RDPB2 had a positive correlation with amide II height (r = 0.78, *P*= 0.01) and tended to have a positive correlation with α–helix height (r = 0.59, *P*= 0.09) and β–sheet height (r = 0.60, *P*= 0.09). The TRDP had a positive correlation with amide I to II height ratio (r = 0.70, *P* = 0.04). RUPA2 had a negative correlation with amide I area (r = -0.69, *P* = 0.04) and tended to have a negative correlation with total amide area (r = -0.66, *P* = 0.05). The RUPB1 had positive correlation with amide I height (r = 0.68, *P* = 0.04) and amide I to II height ratio (r = 0.69, P= 0.04) and tended to have a positive correlation with amide I area (r = 0.65, *P* = 0.06) and total amide area (r = 0.61, *P* = 0.08). The RUPB2 had a positive correlation with amide II height (r = 0.78, *P* = 0.01). The TRUP had a positive correlation with amide I height (r = 0.69, *P*= 0.04) and tended to have a positive correlation with total amide area (r = 0.64, *P*= 0.06).

Table 5.11 shows that there was no correlation between Kd, S, D, U, and BCP NRC when expressed as g/kg of DM primary structure such as Amide I, Amide II, and their ratio and secondary structures such as α–helix, β–sheet, and their ratio (*P* > 0.10). The BCP, when expressed as a percentage, had a positive correlation with α–helix to β–sheet ratio (r = 0.70, *P* = 0.04). The EDCP when expressed as g/kg of DM had a negative correlation with α–helix height (r = -0.78, *P* = 0.01) and β–sheet height (r = -0.76, *P* = 0.02). For degradation kinetics of intestinal digestibility and total tract digestion, dIDP and TDP when expressed as a percentage, were not correlated with

Table 5.9 Predicted truly absorbed metabolizable protein to dairy cows and feed milk value: Comparison total mixed ration with blend-pelleted products (BPP)*

Items	Dietary treatments			SEM	*P*-value	Contrast *P*-value
	Control	BPPCR	BPPCN			Control vs. (BPPCR + BPPCN)
Rumen-synthesized microbial protein truly absorbable in the small intestine (g /kg DM)						
MCP_{RDP}	82.25	84.91	83.47	4.611	0.92	0.99
MCP_{TDN}	94.25	95.72	95.14	0.522	0.25	0.82
AMCP	52.64	53.76	53.42	2.659	0.96	0.95
Rumen-undegradable feed protein truly absorbable in the small intestine (g/kg DM)						
RUP	39.01	36.64	39.39	3.437	0.84	0.73
ARUP	5.98	5.81	6.34	1.052	0.78	0.55
Rumen endogenous protein truly digested in small intestine (g/kg DM)						
ECP	10.93	11.01	11.07	0.048	0.24	0.15
AECP	4.37	4.40	4.43	0.019	0.29	0.19
Total truly absorbed (metabolizable) protein in the small intestine (g/kg DM)						
MP	62.99	63.96	64.19	2.238	0.92	0.81
Degraded protein balance (g/kg DM)						
DPB	-14.45	-13.06	-14.06	5.328	0.98	0.96
Feed milk value (kg milk/kg feed)						
FMV	1.28	1.30	1.31	0.044	0.91	0.77

*Blend-pelleted products based on carinata meal (BPPCR) and canola meal (BPPCN) in combination with pea screenings and lignosulfonate; SEM: standard error of mean; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy; AMCP: truly absorbable rumen-synthesized microbial protein in the small intestine; RUP: rumen-undegradable protein in the small intestine; ARUP: truly absorbable rumen-undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: total metabolizable protein; DPB: degraded protein balance; FMV: feed milk value, kg milk/kg feed.

Table 5.10 Correlation between ruminal degradable and undegradable subfractions of protein of total mixed ration with blend-pelleted products (BPP)* using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.5 and protein molecular structure

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
RDPA2	r	-0.72	-0.04	-0.53	-0.70	0.28	-0.68	0.25	0.18	0.32
(%DM)	*P*-value	0.03	0.93	0.14	0.04	0.47	0.05	0.52	0.64	0.40
RDPB1	r	0.68	-0.27	0.69	0.64	-0.43	0.60	-0.45	-0.41	-0.16
(%DM)	*P*-value	0.04	0.47	0.04	0.06	0.25	0.09	0.22	0.28	0.68
RDPB2	r	0.10	0.78	-0.42	0.12	0.29	0.15	0.59	0.60	-0.12
(%DM)	*P*-value	0.80	0.01	0.26	0.76	0.44	0.70	0.09	0.09	0.76
TRDP	r	0.56	-0.44	0.70	0.52	-0.48	0.47	-0.53	-0.51	0.01
(%DM)	*P*-value	0.11	0.24	0.04	0.16	0.19	0.20	0.15	0.16	0.98
RUPA2	r	-0.71	0.02	-0.55	-0.69	0.34	-0.66	0.26	0.20	0.29
(%DM)	*P*-value	0.03	0.96	0.12	0.04	0.37	0.05	0.50	0.60	0.45
RUPB1	r	0.68	-0.27	0.69	0.65	-0.42	0.61	-0.45	-0.40	-0.16
(%DM)	*P*-value	0.04	0.49	0.04	0.06	0.26	0.08	0.22	0.28	0.67
RUPB2	r	0.12	0.78	-0.39	0.15	0.25	0.18	0.55	0.56	-0.13
(%DM)	*P*-value	0.76	0.01	0.30	0.70	0.51	0.65	0.12	0.12	0.74
TRUP	r	0.69	0.19	0.43	0.65	-0.08	0.64	-0.33	-0.30	-0.31
(%DM)	*P*-value	0.04	0.62	0.25	0.06	0.84	0.06	0.38	0.44	0.42

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP.
RUPA2: ruminally undegraded PA2; RUPB1: ruminally undegraded PB1; RUPB2: ruminally undegraded PB2; TRUP: total ruminally undegraded CP

primary structures and secondary structures ($P > 0.10$; Table 5.12). The IDP, when expressed as g/kg of CP, tended to have a positive correlation with α–helix to *β*–sheet ratio (r = 0.65, $P = 0.06$). Also, IDP when expressed as a percentage had a positive correlation with α–helix to β–sheet ratio (r = 0.73, $P = 0.03$). The TDP when expressed as g/kg of CP had a negative correlation with α–helix height (r = -0.84, $P < 0.01$) and β–sheet height (r = -0.84, $P < 0.01$).

5.4.8. Multiple regression analysis for predicting the ruminal degradation and intestinal digestion using amide spectral profile

Multiple regressions analysis was used to predict the protein profiles and protein subfractions using the Amide spectral features of dietary treatments. The regression analysis for ruminal degradable protein showed that RDPA2 could be predicted from amide I height, taking 52% of the total variance (Table 5.13). The RDPB1 could be predicted from amide I to II height ratio, with 47% of the total difference. TRDP could be predicted from amide I to II height ratio, with 49% of the total difference. For ruminal degradable protein, RUPA2 could be predicted from amide I height, with 50% of the total variance. RUPB1 could be predicted from amide I to Amide II height ratio, with 47% of the total variance. TRUP could be predicted from amide I height, with 48% of the total variance. For degradation kinetics of CP, BCP, when expressed as a percentage, could be predicted from α–helix to *β*–sheet ratio, with 49% of the total variance (Table 5.14). The EDCP, when expressed as a percentage, could be predicted from α–helix to *β*–sheet ratio, with 49% of the total variance. The EDCP, when expressed as g/kg of DM, could be predicted from Amide II height and α–helix to *β*–sheet ratio with 81% of the total variance. For intestinal digestibility, IDP, when expressed as a percentage, could be predicted from α–helix to β–sheet ratio, with 53% of the total variance. The TDP, when expressed as g/kg of DM, could be predicted from α–helix height, with 71% of the total variance.

Table 5.11 Correlation between degradation kinetics of crude protein of total mixed ration with blend-pelleted products (BPP)* with protein molecular structure.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α–helix, β–sheet ratio
Kd (%/h)	r	0.04	0.24	-0.15	-0.20	0.41	-0.16	-0.04	-0.02	-0.15
	P–value	0.93	0.54	0.69	0.60	0.28	0.68	0.91	0.96	0.70
S (%)	r	0.36	0.07	0.23	0.25	0.49	0.30	0.03	0.04	-0.57
	P–value	0.35	0.87	0.55	0.52	0.18	0.43	0.94	0.92	0.11
D (%)	r	0.12	-0.59	0.53	0.27	-0.40	0.23	-0.48	-0.49	-0.10
	P–value	0.77	0.10	0.14	0.49	0.29	0.56	0.19	0.18	0.79
U (%)	r	-0.30	0.43	-0.55	-0.36	0.03	-0.36	0.37	0.36	0.42
	P–value	0.43	0.25	0.12	0.34	0.94	0.34	0.33	0.33	0.26
BCP (%)	r	-0.41	0.19	-0.45	-0.24	-0.47	-0.29	0.46	0.42	0.70
	P–value	0.27	0.63	0.22	0.53	0.21	0.44	0.21	0.26	0.04
BCP NRC	r	-0.27	-0.16	-0.07	-0.12	-0.59	-0.18	-0.33	-0.38	0.52
(g/kg DM)	*P*–value	0.48	0.68	0.85	0.76	0.10	0.64	0.38	0.32	0.15
EDCP	r	0.30	-0.35	0.51	0.20	0.17	0.22	-0.78	-0.76	-0.54
(=RDP, g/kg DM)	*P*–value	0.43	0.35	0.16	0.60	0.66	0.56	0.01	0.02	0.14

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T0: lag time (all zero); S: soluble fraction in the in situ incubation; BCP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP, effectively degraded of crude protein.

Table 5.12 Correlation between degradation kinetics of intestinal digestibility and total tract digestion of total mixed ration with blend-pelleted products (BPP)* with protein molecular structure.

Items		Amide I height	Amide II height	Amide I, II ratio	Amide I area	Amide II area	Amide area	α–helix height	β–sheet height	α, β ratio
%dIDP	r	-0.23	-0.27	-0.10	-0.33	0.28	-0.31	0.18	0.17	-0.02
	P–value	0.54	0.48	0.80	0.38	0.47	0.42	0.65	0.67	0.96
%IDP (g/kg	r	-0.51	-0.34	-0.18	-0.37	-0.55	-0.43	-0.28	-0.34	0.65
CP)	*P*–value	0.16	0.38	0.63	0.32	0.13	0.24	0.47	0.37	0.06
IDP (%)	r	-0.55	0.07	-0.53	-0.42	-0.33	-0.46	0.59	0.54	0.73
	P–value	0.13	0.85	0.14	0.26	0.38	0.21	0.10	0.13	0.03
TDP (g/kg	r	0.18	-0.43	0.46	0.12	0.05	0.12	-0.84	-0.84	-0.39
CP)	*P*–value	0.64	0.25	0.21	0.76	0.91	0.75	0.00	0.00	0.31
TDP (%)	r	0.02	-0.27	0.12	-0.14	0.45	-0.09	-0.04	-0.03	-0.33
	P–value	0.96	0.48	0.76	0.73	0.22	0.81	0.91	0.93	0.38

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein.

Table 5.13 Multiple regression analysis to choose the most important protein spectral parameters to predict protein subfractions, ruminal degradable and undegradable subfractions of protein

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: Y= a + b1 × x1 + b2 × x2 +…….	Model R^2	RSD	*P*-value
Ruminal degradable protein fractions					
RDPA2(%DM)	Amide I height	Y = 6.44 – 11.25 × Amide I height	0.52	0.414	0.03
RDPB1(%DM)	Amide I, II ratio	Y = 3.82 + 0.74 × Amide I, II ratio	0.47	0.717	0.04
TRDP(%DM)	Amide I, II ratio	Y = 9.73 + 0.39 × Amide I, II ratio	0.49	0.376	0.04
Ruminal undegradable protein fractions					
RUPA2(%DM)	Amide I height	Y = 0.87 - 1.54× Amide I height	0.50	0.057	0.03
RUPB1(%DM)	Amide I, II ratio	Y = 1.53 + 0.30 × Amide I, II ratio	0.47	0.285	0.04
TRUP(%DM)	Amide I height	Y = 3.12 + 9.03× Amide I height	0.48	0.333	0.04

RSD: residual standard deviation; PA2: rapidly degradable true protein; PB1: moderately degradable true protein; RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RUPA2: ruminally escaped PA2; RUPB1: ruminally escaped PB1; TRUP: total ruminally undegraded CP.

5.1. Discussion

Canola meal is widely used as a protein source for lactating dairy cows rations in North America. In recent years, carinata meal, a new protein feed has been introduced in feedlot diets and dairy heifers (Guidotti 2018; Rodriguez-Hernandez et al. 2108). However, there is no study on the effect of feeding this new feed in lactating cows in terms of ruminal digestion, ruminal fermentation characteristics.

Carinata and canola meal have been shown to have high ruminal digestion of protein (Wright et al., 2010; Xin et al., 2013; Ban et al., 2018); thus it is essential to slow down their ruminal digestion in the rumen by applying heat treatment (pelleting or extrusion) or using feeding additives (i.e., lignosulfonate and tannins). A previous study by Guevara-Oquendo et al. (2018) reported that blend-pelleted products based on carinata meal would exhibit higher nutritive value relative to blend-pelleted products based on canola meal.

Thus, in the current study, one pellet based on carinata meal (lignosulfonate 4.8% + carinata meal 71.4% + pea screenings 23.8%) was selected to study its effect on ruminal digestion using in situ technique relative to pelleted products based on canola meal (lignosulfonate 4.8% + canola meal 71.4% + pea screenings 23.8%). It was found that heat treatment and the addition of lignosulfonate can reduce the proportion of ruminal degradable protein, thereby increase the available essential amino acids to the mammary gland for milk synthesis (Wright, 1998; Wright et al., 2005).

The CNCPS model was updated to predict the ruminal digestion of CHO and protein. The updated system uses different degradation rates and passage rates. The PA1 and PA2 have higher passage and degradation rates, while the PB2 has a slow degradation rate and passage rate similar to CB3 in carbohydrates fractions (Higgs et al., 2015). The RUP tended to be higher in BPPCN

Table 5.14 Multiple regression analysis to choose the most important protein spectral parameters to degradation kinetics of intestinal digestibility and total tract digestion

Predicted variable (Y)	Variable selection (variables left in the model with $P < 0.05$)	Equation prediction: $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots$	Model R^2	RSD	*P*-value
Degradation kinetics of CP					
BCP (%)	α, β– ratio	Y = –36.70 + 77.54 × α, β– ratio	0.49	10.039	0.04
%EDCP	α, β– ratio	Y = 136.70 – 77.54 × α, β– ratio	0.49	10.039	0.04
EDCP (=RDP, g/kg DM)	Amide II area, α–helix height	Y = 36.16 +44.13 Amide II area – 278.59 × α–helix height	0.81	28.571	<0.01
Intestinal digestibility and total tract digestion of CP					
IDP, %	α, β– ratio	Y = -29.84 + 60.29 × α, β– ratio	0.53	7.804	0.03
TDP, g/kg DM	α–helix height	Y = 146.92 – 250.64 × α–helix height	0.71	37.754	<0.01

RSD: residual standard deviation; BCP: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded of crude protein; IDP: intestinal digested crude protein; TDP: total digested crude protein; DM: dry matter

than the other diets, while the TRDC tended to be lower in the BPPCN compared with different diets. The lower TRDC would be due to the higher lignin content in BPPCN reported in the previous chapter. The higher TRDC would, in turn, enhance the ruminal bacteria growth and increase the MCP in dairy cows.

There was no effect of different diets on in situ ruminal digestion of DM, CP, starch, and NDF. Adding lignosulfonate to BPPCN or BPPCR did not increase the bypass protein as expected. These findings are not in agreement with an earlier study by Wright et al. (2005) who reported improvement in N bioavailability in dairy cows and milk production. The higher N utilization is due to improving the bypass protein and reduction in urinary N excretion in dairy cows. Adding lignosulfonate to BPPCR and BPPCN diet did not influence the apparent digestibility of nutrients. These findings are in not in line with Wright et al. (2005) or Neves et al. (2009), who found the RDP and total tract digestibility decreased after adding lignosulfonate to canola meal. The TMR based on BPPCR exhibited similar BCP to other diets (averaging 38 %CP). In contrast, Guevara-Oquendo (2018) reported a lower BCP in BPPCR relative to BPPCN (50 vs. 63%CP). Carinata meal was also reported to contain lower BCP than canola meal in previous studies (25 vs. 40% CP; Xin and Yu, 2014; Ban et al., 2018). Using the omasal sampling technique to evaluate the ruminal digestion and omasal nutrient in beef cows, Guidotti (2018) reported similar ruminal DM, OM, NDF, and CP for feedlot fed diets based on canola meal or carinata meal. The same author also reported similar RDP (averaging 65% of N intake). To my knowledge, there is no study on ruminal digestion and intestinal digestion in lactating cows fed TMR based on carinata meal versus canola meal. The results in this study indicate that BPPCR has the same digestion behavior as BPPCN in dairy cows.

Using the in vitro technique to evaluate the intestinal digestion of dietary treatments, the result in the current study showed that predicted intestinal digestion of protein was the same for all diets. The total ruminal and intestinal digestion of DM, CP, and starch were similar for all diets. These results are in agreement with the total tract digestibility results for the same dietary treatment in lactating ration showed in the previous Chapter. The MP content of a feed is the total protein content that contributes to milk production. The total MP in the NRC model is the summation of AECP, ARUP, and AMCP (NRC, 2001). The results of the current study showed that the MP content was the same for all dietary treatments (averaging 74 g/kg DM). These results are not in line with Guevara-Oquendo (2018), who reported high MP values for BPPCR relative to BPPCN (231 vs. 163 g/kg DM). The findings in the current study would explain the non-significant results found in production performance in the previous Chapter.

The cows fed BPPCN exhibited lower ruminal pH and longer duration of rumen pH < 5.8. These findings are in line with Krizsan et al. (2017) who found a linear reduction in rumen pH after increasing the level of canola meal in the diet. The drop in ruminal pH and longer duration time of pH under 5.8 in BPPCN would be due to the relatively low ruminal ammonia concentration and higher lignin content in this diet which might increase the passage rate. The ruminal VFA was altered by feeding the BPPCN, where the total VFA and propionic acid were significantly lower in BPPCN than the control diet. The lower ruminal fermentation may be due to the lower TRDC in BPPCN or due to the lower ruminal pH which decreases the ruminal activity of cellulolytic bacteria and amylolytic bacteria and increase the activity of *Lactobacilli* bacteria (Wells et al., 1997). A significant increase in the population of ruminal lactobacilli is a common attribute to both acute and subacute ruminal acidosis (Goad et al., 1998). The results in the current study are

not in line with the previous research by Guidotti (2018) who did not find any difference in ruminal fermentation profile between beef cows fed a diet based on carinata meal and canola meal.

The study on the association between molecular structures of different blend-pelleted products based on carinata meal and canola meal and the metabolic characteristic of protein in dairy cows showed that there was a significant correlation between the Amide I height and Amide I area and TRUP. It has been observed that the changes in the ratio of α-helix to β-sheet ratio could induce alterations in molecular protein makeup (Yu, 2005). The high proportion of β-sheet structure could limit the access of gastrointestinal digestive enzymes, which results in a low protein value (Yu, 2005). In this current study, there was a significant correlation between intestinal digested crude protein and α-helix and β-sheet ratio.

5.2. Conclusions

The blend-pelleted products based on carinata meal as a new co-product from bio-fuel processing industry is equal to the other pelleted products based on canola meal as a protein source for dairy cattle. Carinata meal as the pellet is similar to the pelleted products based on canola meal in the affecting nutrients utilization, rumen fermentation, and rumen degradability in lactating dairy cows. The molecular spectroscopy can detect inherent structural characteristics in the blend-pelleted products based on different bio-energy co-products. The molecular structural features related to the protein region are highly associated with the protein utilization in dairy cows. It is safe to use carinata meal as an alternative source of protein for dairy cows because it contains a high proportion of protein and without any negative impacts in the production perforce for high producing dairy cows.

6. GENERAL DISCUSSION, OVERALL CONCLUSIONS, AND IMPLICATIONS

A new co-product from bio-fuel processing, carinata meal, has been reported to have a high nutritional value. However, the metabolic characteristics, lactation performance of newly developed carinata meal, a co-product from bio-oil processing, as a feed ingredient has not been investigated in high producing dairy cows. Furthermore, there is no report on the association between the molecular structure features of the amide region and lactation performance and nitrogen balance of high producing dairy cows. This thesis research was conducted to study the effect of feeding different blend-pelleted products based on traditional bio-oil co-product (canola meal) and the new co-product from bio-fuel processing (carinata meal) in molecular structural features related to amide region, lactation performance, metabolic characteristics, and nitrogen balance in dairy cows in comparison with a typical protein blend-pelleted products (blend of canola and soybean meal).

Trial 1 (Chapter 3) was designed to determine the molecular structure features related to amide region and to quantify the relationship between structural features and protein bioavailability of blend-pelleted products based on canola meal and the new bio-fuel co-product (carinata meal) with different proportions of pea screenings and lignosulfonate compound in dairy cows. In recent years, the FTIR spectroscopy has been established to quantitatively estimate the molecular make-up of feed protein (Yu, 2007a). Using FTIR can reveal information about the molecular structure of feed, i.e., the amide I and II bands (Damiran and Yu, 2011; Peng et al., 2014). The amide I to II ratio of BPPs was highest in the co-products based on carinata meal and due to the low inclusion level of pea screenings. The high ratio of amide I to II is influenced by improving the metabolizable protein supply in dairy cows (Doiron et al., 2009; Liu et al., 2012; Yu, 2006). The secondary structure of amid region was not influenced by co-product or pea

screening levels in BPPS. The lack of significant effect of co-products and pea screening is attributed to the similarity in processing methods. The principal component analysis (PCA) was conducted to reduce the number of variables and to detect the relationship among different treatments. The results showed that most of the BPPs based on canola meal were separated from the BPPs based on carinata meal by PC2 which accounted for 5% of the variance. The loading point plots were used to determine the essential regions responsible for the clustering. The amide region at ca. 1650 cm^{-1} of PC2 was the most important parameter for discriminating the BPPs.

The trial 2 (Chapter 4)was conducted to examine the effects of feeding the newly developed blend-pelleted products based on carinata meal (BPPCR) or canola meal (BPPCN) on the production efficiency of high producing dairy cows; and to study the association between the molecular structure features related to amide region and nitrogen utilization and the production efficiency in high producing dairy cows. The results in this study showed that all the dietary treatments were similar in milk yield, milk components yield, and feed efficiency in dairy cows. All diets in the present study were formulated to have the same protein content for understanding the protein utilization in dairy cows. Our results are in line with Guidotti (2018), who found that feeding carinata meal did increase the growth performance of growing beef cows in comparison with canola meal. Another study by Rodriguez-Hernandez and Anderson (2018) also reported no significant effect of feeding carinata meal on the growth performance of growing dairy heifers. There was no beneficial effect of adding lignosulfonate to carinata meal or canola meal in improving the production performance of dairy cows relative to the control diet. Adding lignosulfonate to BPPCR and BPPCN diet did not influence the apparent digestibility of nutrients. These findings are in not in line with a previous study by Wright et al. (2005), who found that treating canola meal with lignosulfonate decreased the digestibility of protein. The lack effect of

adding lignosulfonate to canola meal or carinata meal is attributed to the lack effect of this feed additive on the ruminal degradable protein.

Gross milk revenue was the same for all dietary treatments (averaging $30.8 per cow/day). The income-over-feed cost was increased by about $1.07 per cow/day in the blend-pelleted products based on carinata meal relative to the blend-pelleted product based on canola meal. The molecular structure features related to the amide region was used to study the association between the amide region and nitrogen balance in high producing dairy cows. The results showed that the urinary N excretion, fecal N excretion, and total N excretion were significantly correlated with the amide region. The milk nitrogen content found to be significantly associated with the Amide I height and the secondary structure of Amide I region.

Trial 3 (Chapter 5) was carried out to evaluate the effect of feeding the newly developed blend-pelleted products based on carinata meal or canola meal in combination with peas screenings and lignosulfonate on ruminal fermentation characteristics, ruminal degradability, and intestinal digestion in high producing dairy cows. The blend-pelleted products based on canola meal exhibited low ruminal pH and total VFA relative to control diet. There was no significant effect of adding dietary treatments on ruminal and intestinal digestion of DM, CP, and NDF in dairy cows. Using the omasal sampling technique to evaluate the ruminal digestion and omasal nutrient in beef cows, Guidotti (2018) reported similar ruminal DM, OM, NDF, and CP for feedlot fed diets based on canola meal or carinata meal. The same author also reported similar RDP (averaging 65% of N intake). To my knowledge, there is no study on ruminal digestion and intestinal digestion in lactating cows fed TMR based on carinata meal versus canola meal. The results from this study indicate that BPPCR has the same digestion behavior as BPPCN in dairy cows.

In conclusion, the blend-pelleted products based on carinata meal as a new co-product from bio-fuel processing industry is equal to the similar pelleted products based on canola meal as a protein source for dairy cattle without limiting the performance of high producing dairy cows. Adding lignosulfonate to the blend-pelleted products did not improve the N utilization in dairy cows. The molecular spectroscopy approach can identify structural characteristics in dietary treatments based on different bio-energy co-products. The molecular structural features related to the amide region are highly associated with the N utilization in dairy cows. Further research is warranted to determine the influence of other processing methods to carinata meal on degradability and to compare the production performance of dairy cows fed carinata meal to those fed other traditional protein sources such as distillers and soybean meal in high producing dairy cow.

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APPENDIX

Table A.1. Summary statistics values for the chemical profile, protein sub fractions and predicted energy profiles for the blend-pelleted products* data from (Guevara-Oquendo, 2017)

Items	Mean (n = 16)	STD	Minimum	Maximum
Basic chemical profile				
CP (g/kg DM)	392.2	38.0	336.3	457.9
NDICP (g/kg CP)	99.2	37.4	55.5	150.8
ADICP (g/kg CP)	23.2	9.2	10.6	36.8
SCP (g/kg CP)	307.8	66.4	185.1	427.0
NPN (g/kg CP)	32.75	4.15	24.66	41.41
Predicted energy values by NRC				
tdCP (g/kg DM)	388.6	38.2	332.1	455.2
TDN_{1x} (g/kg DM)	753.7	28.2	709.7	793.2
ME_{p3x} (g/kg DM)	2.98	0.12	2.80	3.15
NE_{Lp3x} (g/kg DM)	1.91	0.09	1.78	2.03
Protein subfractions (CNCPS v. 6.5)				
PA2 (g/kg CP)	347.7	74.7	208.4	479.7
PB1 (g/kg CP)	553.1	53.1	464.8	655.4
PB2 (g/kg CP)	76.0	45.8	28.0	135.2
PC (g/kg CP)	23.2	9.2	10.6	36.8
Ruminal degradation kinetics of CP				
Kd (%/h)	9.17	2.38	5.39	17.89
S (%)	17.53	2.84	9.79	23.12
D (%)	70.82	4.06	62.04	77.43
U (%)	11.65	5.10	4.36	21.93
BCP (%)	40.33	6.08	32.01	52.35
EDCP (%)	59.67	6.08	47.65	67.99
Intestinal digestion of CP				
dIDP (%)	73.56	5.44	63.07	83.37
IDP (%)	29.92	6.34	21.20	40.65

Table A.1. *Cont'd.* Summary statistics values for the chemical profile, protein sub fractions and predicted energy profiles for the blend-pelleted products* data from (Guevara-Oquendo, 2017)

Items	Mean (n = 16)	STD	Minimum	Maximum
Predicted values of potential nutrient supply to dairy cattle (g/kg DM)				
MCP_{RDP}	197.91	19.45	6333.0	171.2
MCP_{TDN}	89.95	3.30	2878.0	84.7
AMCP	57.57	2.11	1842.0	54.2
RUP	159.33	35.12	5099.0	111.6
ARUP	118.42	32.52	3789.0	79.5
MP	180.19	33.78	5766.0	140.3
DPB	126.70	25.49	4054.0	91.0
Feed Milk Values (kg milk/kg DM)				
FMV	3.39	0.78	108.3	2.1

*Eight blend-pelleted based on carinata meal and canola meal in combination with different level of lignosulfanate and pea screenings ; STD : standard deviation ; CP : crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non–protein nitrogen ; tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one time maintenance; $ME_{3\times}$: metabolizable energy at production level of intake (3×); $NE_{L3\times}$: net energy for lactation at production level of intake (3×); PA2: soluble true protein; PB1: insoluble true protein; PB2: fiber–bound protein; PC: indigestible protein; Kd: degradation rate; S: soluble fraction in the in-situ incubation; D: potentially degradable fraction; U: undegradable fraction; BCP: bypass crude protein; EDCP: effectively degraded of crude protein; dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digestion of crude protein; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy (discounted TDN); AMCP: truly absorbed rumen–synthesized microbial protein in the small intestine. RUP: ruminally undegraded feed CP, calculated according the formula in NRC–2001 dairy model; ARUP: truly absorbed rumen undegraded feed protein in the small intestine; MP: metabolizable protein (true protein that is digested postruminally and the component amino acid absorbed by the intestine); DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy–TDN available for microbial fermentation in the rumen; FMV: feed milk value (based on metabolic characteristics of protein predicted by NRC 2001

Table A.2. Summary of the chemical and nutrient composition for the blend-pelleted products* (Guevara-Oquendo, 2017)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8
Basic chemical								
DM (%)	87.9	88.3	88.9	88.9	88.0	88.2	88.9	89.1
Ash (%DM)	5.9	6.5	7.2	7.7	5.6	6.1	6.9	7.3
EE (%DM)	1.6	1.4	1.5	1.2	2.8	2.0	3.1	2.4
FA (%DM)	0.6	0.4	0.5	0.2	1.8	1.1	2.1	1.4
OM (%DM)	94.1	93.6	92.8	92.3	94.4	93.9	93.1	92.7
Protein profile								
CP (%DM)	38.8	36.4	45.0	43.1	35.9	33.7	41.9	39.0
NDICP (%CP)	13.0	13.1	14.4	13.4	6.0	6.0	6.7	6.8
ADICP (%CP)	1.4	1.6	1.5	1.4	2.7	3.0	3.3	3.6
SCP (%CP)	27.3	28.0	25.4	23.7	38.7	34.7	37.1	31.4
NPN (%CP)	34.0	35.2	29.7	29.8	36.6	37.9	29.0	29.8
Carbohydrate profile								
CHO (%DM)	53.7	55.7	46.3	48.0	55.7	58.2	48.1	51.3
ST (%DM)	25.4	25.3	14.6	13.3	26.8	25.8	14.5	14.8
Sugar (%DM)	6.5	6.5	7.2	7.7	6.3	6.7	7.2	7.8
NDF (%DM)	19.3	18.4	21.0	19.5	20.3	19.7	22.6	21.5
ADF (%DM)	9.3	9.2	9.4	8.9	12.6	12.6	15.2	14.9
ADL (%DM)	1.4	1.6	1.9	1.8	4.9	4.7	6.7	6.6

*Blend-pelleted products based on carinata meal and canola meal in combination with pea screenings and lignosulfonate; BPP1: lignosulfonate 0 % DM + carinata meal 50 % DM + pea screenings 50.0 % DM.; BPP2: lignosulfonate 4.8 % DM + carinata meal 47.6 % DM + pea screenings 47.6 % DM; BPP3: lignosulfonate 0 % DM + carinata meal 75 % DM + pea screenings 25 % DM; BPP4: lignosulfonate 4.8 % DM + carinata meal 71.4 % DM + pea screenings 23.8 % DM; BPP5: lignosulfonate 0 % DM + canola meal 50 % DM + pea screenings 50.0 % DM; BPP6: lignosulfonate 4.8 % DM + canola meal 47.6 % DM + pea screenings 47.6 % DM; BPP7: lignosulfonate 0 % DM + canola meal 75 % DM + pea screenings 25 % DM; BPP8: lignosulfonate 4.8 % DM + canola meal 71.4 % DM + pea screenings 23.8 % DM; DM: dry matter; EE: ether extract; FA: fatty acid; OM: organic matter; CP : crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non–protein nitrogen; CHO: carbohydrate; ST: starch; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: lignin.

Table A.3. Summary for the chemical profile of the feed ingredients of blend pelleted products that used in this project* (Guevara-Oquendo, 2017)

Items	Pea screenings	Carinata meal	Canola meal
DM (%)	85.7	90.1	89.9
Ash (%DM)	3.1	8.5	8.1
EE (%DM)	1.6	1.3	3.1
FA (%DM)	0.6	0.3	2.1
OM (%DM)	96.9	91.5	91.9
CP (%DM)	22.5	52.3	49.8
NDICP (%CP)	4.0	16.9	6.5
ADICP (%CP)	0.7	1.5	3.3
SCP (%CP)	56.4	26.4	38.0
NPN (%CP)	29.2	24.2	28.1
CHO (%DM)	72.8	37.9	39.0
ST (%DM)	48.0	1.9	2.0
Sugar (%DM)	3.4	7.4	7.5
NDF (%DM)	19.0	23.2	24.7
ADF (%DM)	7.7	9.3	17.3
ADL (%DM)	1.0	2.2	8.7

DM: dry matter; EE: ether extract; FA: fatty acid; OM: organic matter; CP : crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non–protein nitrogen; CHO: carbohydrate; ST: starch; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: lignin.

Table A.4. The cost of feed ingredients that used in this project

Ingredient	Unit cost ($)
Barley silage	45.00
Alfalfa hay	160.00
Steam-flaked barley	230.00
Bypass palmitic acid	1675.00
Soybean meal	548.00
Peas	265.00
Dynk	980.00
Sodium bicarbonate	625.00
Tallow	800.00
Limestone ground	185.00
Mineral premix	2094.75
Ameribond	977.00
Canola meal	380.00
Carinata meal	380.00
Peas screenings	200.00
Dynmate	1100.00
Calcium propiontate	1100.00