REVEALING THE ASSOCIATION OF FEED INTRINSIC MOLECULAR STRUCTURE WITH NUTRIENT SUPPLY TO ANIMALS FROM FEEDSTOCKS AND CO-PRODUCTS FROM BIO-OIL PROCESSING USING ADVANCED MOLECULAR SPECTROSCOPY TECHNIQUES

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By

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ABSTRACT

Canola (*Brassica napus)* is a crop grown primarily for oil extraction from the seeds. However, this process generates a co-product called canola meal, that is rich in protein. Modifications in the processing of the seeds can affect the meal. The general objective of this research was to reveal the association of intrinsic molecular structures with the nutrient supply to dairy cows from canola seeds and meals from two countries using an advanced vibrational molecular spectroscopy technique, the Fourier transform infrared – Attenuated Total Reflectance (FTIR-ATR).

Chapter 1 brings a general introduction of this thesis. And chapter 2 contains the literature review that demonstrates the reasoning and some methods considered when developing this project. Following these, from chapter 3 to chapter 6 each study with its respective results is discussed, and chapter 7 summarizes the whole project. All references are presented on chapter 8 and extra tables and information are brought in the final chapter, chapter 9.

In chapter 3, the chemical and nutrient profiles of canola seeds and meals from Canada and China were evaluated. The results showed that DM (dry matter) was higher on Canadian canola meals (89.96 vs. 88.55%, $P<0.001$) and CP (crude protein) was higher in Chinese meals (43.04 vs. 41.87% DM, $P=0.003$), but only DM was higher in Canada's seeds (93.10 vs. 92.28%, $P=0.008$). Chinese meals presented higher tdNDF ($P<0.001$) and tdCP ($P<0.001$), and lower tdNFC ($P=0.006$) than Canada's. Only tdNDF of canola seeds was higher in Canada ($P=0.023$). The soluble fraction (PA2) was higher ($P<0.001$) in meals from China and the slowly degradable fraction (PB2) was higher ($P<0.001$) in meals from Canada. Chinese meals and seeds showed higher content of water-soluble carbohydrates (CA4) ($P=0.040$ and $P=0.022$, respectively). And Canadian meals presented higher soluble (CB2) and indigestible (CC) fiber contents ($P=0.010$ and $P<0.001$). These suggest that although few differences were observed, different procedures in crushing plants affect the meals.

Chapter 4 examines the ruminal degradation and intestinal digestibility and provides a characterization of nutrient supplies. From these studies, results showed that the rumen undegradable fraction (U) was higher in Canadian meals ($P=0.025$) and the rumen degradable

fraction (D) was higher in Chinese meals (P=0.016). Also, the hourly degradation of CP was higher in Chinese canola meals on 24 (P=0.042) and 48 hours (P=0.040) of incubation. The *in vitro* intestinal digestibility showed that the total digestible dry matter and the intestinal digestibility of protein of the canola meals from China were higher (P=0.018 and P=0.016, respectively) than from Canada. The feed milk value (FMV) was determined according to the NRC 2001, DVE/OEB model and based on the energy and no differences were observed for seeds or meals between countries on either method (P>0.05). These results propose that the ruminal and intestinal performance of canola meals and seeds from different companies and countries is similar, as well as the nutrient supply to dairy cows.

Chapter 5 is the molecular spectroscopy study of protein and carbohydrate-related structures from canola seeds and meals. Chinese meals showed higher peak heights for total carbohydrate on peaks 3 and 4 (TC3, TC4), cellulosic compounds (CEC), structural carbohydrates (STC2, STC3, and STC4), and areas for TC, CEC, and STC (P<0.05). Canadian canola seeds presented higher peaks for TC1, TC2, TC3, TC4, CEC, STC2, STC4, and TC area (P<0.05), while the ones from China showed a higher peak for SCT1 (P=0.033). Regarding the protein-related structures of canola seeds, they showed no differences between countries (P>0.05). However, the Chinese meals presented higher amide I height; α-helix and β-sheet heights and their ratio; and amide and amide I areas (P<0.05). Principal Component Analysis (PCA) done on the FTIR-ATR analysis was not able to completely differentiate samples from different countries or companies. These results suggest that the canola seeds and meals processed in Canada are comparable to those processed in China.

Chapter 6 describes the relationship between the molecular structures spectra features of canola seeds and meals and nutrient utilization and availability to dairy cows. The results from the correlation study showed that the area of structural carbohydrate (STCA) commonly appears to be related to meals' characteristics and total carbohydrate area (TCA) to features of the seeds. The amide region showed a strong relationship with nutritional characteristics of both seeds and meals. These results indicate that the carbohydrate and protein structures studied with FTIR-ATR are related to canola seeds and meals' chemical and nutrient profiles, as well as rumen degradable and intestinal digestibility characteristics.

In conclusion, canola seeds processed in crushing plants in Canada mostly presented the same characteristics and behaviors as the seeds processed in China; although canola meals presented some differences in the chemical and nutrient profiles, they provide similar nutrient supply and utilization to dairy cows; and the FTIR-ATR technique applied on the samples to study protein and carbohydrate-related structures proved to an efficient method to predict chemical, degradable and digestible characteristics of canola meals and seeds.

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LIST OF ABBREVIATIONS

ADF: Acid Detergent Fiber

ADICP: Acid Detergent Insoluble Crude Protein

ADL: Acid Detergent Lignin

AOAC: Association of Official Agricultural Chemists

BCP: By-pass Crude Protein

BDM: By-pass Dry Matter

CHO: Carbohydrate

CLA: Cluster Analysis

CNCPS: Cornell Net Carbohydrate and Protein System

CP: Crude Protein

D: Insoluble but Potentially Degradable Fraction in Nylon Bag Incubation

DE: Digestible Energy

DM: Dry Matter

dRUC: Digestion of Rumen Undegradable Carbohydrate

dRUP: Digestible Rumen Undegradable Protein

DVE: True Protein Digested in the Small Intestine

EDCP: Endogenous Crude Protein

EDDM: Endogenous Dry Matter

EE: Ether Extract

FTIR: Fourier-Transform Infrared Spectroscopy

FTIR-ATR or ATR/FTIR: Fourier-Transform Infrared Spectroscopy – Attenuated Total Reflectance

IDP: Intestinal Digestion of Total Crude Protein

Kd: Digestion Degradation Rate

Kp: Passage Rate

ME: Metabolizable Energy

MEg: Metabolizable Energy for Gain

MEm: Metabolizable Energy for Maintenance

MP: Metabolizable Protein

NDF: Neutral Detergent Fiber

NDICP: Neutral Detergent Insoluble Crude Protein

NDIP: Neutral Detergent Insoluble Protein

NDS: Neutral Detergent Soluble

NE: Net Energy

NEL: Net Energy for Lactation

NFC: Non-Fiber carbohydrate

NPN: Non-Protein Nitrogen

NRC: National Research Council

OEB: Degraded Protein Balance

PCA: Principal Component Analysis

RDC: Rumen Degradable Carbohydrate

RDDM: Rumen Degradable Dry Matter

RDP: Rumen Degradable Protein

RDP: Rumen Degradable Protein

RUC: Rumen Undegradable Carbohydrate

RUDM: Rumen Undegradable Dry Matter

RUP: Rumen Undegradable Protein

S: Soluble Fraction in Nylon Bag Incubation

SCP: Soluble Crude Protein

TCA: Trichloroacetic Acid

tdCP: Total Digestible Crude Protein

tdFA: Total Digestible Fatty Acids

TDN: Total Digestible Nutrients

tdNDF: Total Digestible Neutral Detergent Fiber

tdNFC: Total Digestible Non-Fiber Carbohydrate

TRUCHO: Total Rumen Undegradable Carbohydrate

TRUP: Total Rumen Undegradable Protein

T0: Lag Time

U: Undegradable Fraction in Nylon Bag Experiments

VIF: Variance Inflation Factor

1. GENERAL INTRODUCTION

The human population increases every year. Therefore, the demand for food in general also increases. Animal products such as milk, meat, and eggs play an essential part on human diet, and many animal producers struggle to provide enough products with a quality that meets the standard for market. Producers and researchers are constantly searching for methods to improve the production and reduce the costs without affecting the quality of the final products. The search for ideal feed supplements that can provide the necessary nutrients to the animals, that will not overload the industry, and can reduce costs is present in every department, university, and farm around the world.

In Canada, the production of canola is abundant due to its adaptability to the growing conditions of the prairies and provides great quality oil for human consumption (Eskin, 2016). However, the process to extract the oil from the canola seeds generates a residue, now recognized as a co-product due to its high feed quality, called canola meal. While the seeds can contain up to 43% oil, the meals can contain a minimum of 35% of protein, which aligned with the amino acids profile makes canola meal a great source of protein specially for dairy rations. The canola produced nowadays is also called “double-zero” for having close to zero levels of erucic acids in the oil and glucosinolates in the meals, both compounds when in high levels reduce the palatability of the products (Eskin, 2016). So, researchers continue to study the quality and effects of canola meal on animal feeds, and how its quality can be improved through processing so even better results can be obtained from the animals’ production.

Various methods of analyses have been used to assess the nutrient profile, rumen degradability, intestinal digestibility, and production performance of feed ingredients and full rations. On this project the objective was to compare the characteristics of canola seeds and meals from five batches from five different crushing companies in Canada and from five different

crushing companies in China. The chemical and nutrient profiles, ruminal degradability, and intestinal digestibility, as well as performance prediction, and protein and carbohydrate spectral structures in relation to nutrient utilization and availability were analyzed and compared between companies and countries.

Starting with following chapter, the literature review provides information about canola production and processing, and about the available methods of analysis, including others that are not used in this project, concluding with the hypotheses and objectives of this project. The third chapter provides the results and a discussion about the chemical and nutrient profiles of the canola seeds and meals analyzed in this project using AOAC standard methods of analyses, the NRC model, and the CNCPS system. The fourth chapter communicates the results from the ruminal degradation (*in situ* procedure) and from the intestinal digestion (three-step *in vitro* procedure) studies. The fifth chapter reveals the intrinsic molecular structures related to proteins and carbohydrates of the samples studied using the ATR-FTIR spectroscopic technique. The sixth chapter shows the relationships between the molecular structures of canola seeds and meals and their nutrient utilization and availability to dairy cows. And finally, the seventh chapter brings a brief general discussion about the results of all the studies performed in this project.

2. LITERATURE REVIEW

2.1. Canola production and use in Canada

2.1.1. Canola production in Canada

Canada is the largest producer and exporter of canola worldwide. Canada's inspection system for canola is considered world-class, which assures the Canadian canola the high-quality label. Canadian canola is an important source of income to the country's economy, contributing almost $27 billion dollars, in which $11 billion come from the export of canola products to more than 50 markets worldwide (CANADA, 2019).

One of Canada's biggest markets is China, which alone accounts for about 40% of all the canola oil, seed, and meal exports. Canola seeds exported to China in 2018 contributed $2.7 billion dollars to the Canadian economy (Canola Council of Canada (CCC), 2019).

According to the 2020 Annual Report published by Canola Council of Canada (n.d.a), in 2020, canola and rapeseed production in the world reached 27.3 million metric tons. Of these, 18.7 million metric tons correspond to the canola produced in Canada.

2.1.2. Canola features

Canola was developed to be a seed higher in oil and lower in erucic acid and glucosinolates contents (Canolamazing, n.d.). It originated from breeding of varieties of Brassica napus in Canada in 1974. The first low erucic and low glucosinolates variety was developed by a team led by Dr. Stefansson at the University of Manitoba and received the name of "Tower". This variety

was licensed in February of 1974 and in 1978, the industry adopted the name “canola” (which stands for Canadian oil) to differentiate this product when compared to other rapeseeds produced around the world (CCC, n.d.b). Since its introduction to the producers, canola has become a major crop in western Canada (Theodoridou and Yu, 2013). According to Assadi, Janmohammadi, Taghizadeh, and Alijani (2011), over the past 20 years, the global production of canola has exceeded the production of peanuts, sunflower seed, and cottonseed.

Whole oilseeds are added to the diets of ruminants to bring an increment of high-quality protein and a higher energy density, while avoiding the rancidification that occurs when fat is added during the mixing of ration in the farm (Hussein, Merchen, and Fahey, 1996a). Whole canola seeds have been reported as being heavily explored since 1996 mainly because of its high content of lipids (about 55%, mostly composed of long chain fatty acids) and protein (about 20.6%) (Aldrich, Merchen, Drackley, Fahey, and Berger, 1997).

Assadi et al. (2011) analyzed three varieties of canola seeds produced in Iran and had similar results with the mean of crude protein and ether extract being, 20.3% and 48.7%. Their varieties also presented higher proportions of long chain fatty acids, which is an important source of fat to be introduced to the animals’ diets, aiming the gain in quality of the milk and meat (Hussein et al., 1996a, 1996b).

2.2. Rapeseed production and canola import in China

2.2.1. Rapeseed production in China

China has been breeding varieties of rapeseeds to reach the same low levels of erucic acid and glucosinolates as canola. They have developed rapeseed varieties with less than 3% erucic acid and equal or less than 35µmol/g of glucosinolates (Hu et al., 2017). This is a great advance, but canola still has lower levels of both classes of compounds. China imports a significant amount of canola products to accommodate their high internal demand.

China produces rapeseed in large scale; however, their demand is over their capacity of production. Also, a reduced availability of labor and deficiency of arable land have been forcing producers to be more mechanized to improve their productions by reducing labor and maximizing the use of space available (Hu et al., 2017). China grows rapeseed in a triple-crop system, which consists of 2 crops of rice and 1 crop of rapeseed, thus only 165 days in production of rapeseed per year per farm. Although there is pressure to supply enough rapeseed to meet the demand, there are only a few large mechanized rapeseed farms in China. Most of the farms are still small and seeded and harvested by hand (Whetter, 2018).

2.2.2. Export of canola seed to China from Canada

According to a market overview of China (Agriculture and Agri-Food Canada, 2021), in 2019, China was the most populous country, the world's second-largest economy, and the third-largest importer of seafood and agri-food products, with a gross domestic product (GDP) increase of US$11.5 trillion. With its rising population and urbanization, China is one of Canada's most important export markets. China imported the equivalent to Can$198.9 billion of agri-food and seafood products in 2019, growing 7.6% from 2015 to 2019. However, there was a 38.0% decline in the import from Canada of oilseeds, canola oil, grains, cereals, vegetables, and pulses in 2019.

The 2020 Annual Report published by Canola Council of Canada (n.d. a), demonstrated that China imported 2.6 million metric tons of canola seed; 1.1 million metric tons of canola oil; and 1.5 million metric tons of canola meal; of the 11.8 million metric tons of canola seed; 3.4 million metric tons of canola oil; and 5 million metric tons of the canola meal exported from Canada in 2020. The above confirms that China is an important market for canola products.

2.3. Oilseed processing

Canola seeds are primarily used in the industry for oil extraction but they are also used as a feed ingredient for ruminant rations. This process of extracting oil from canola seeds produces

an intermediate product called canola presscake that is the oil-extracted seed that has been cooked, flaked and expeller-processed, and only after being solvent-extracted or toasted it produces a final residue called canola meal, that is very low in fat content but rich in protein (Theodoridou and Yu, 2013).

2.3.1. Bio-oil processing procedures and conditions

The processing of canola seeds to extract oil is composed of seed cleaning; pre-conditioning and flaking; seed cooking; pressing the flake; solvent extraction; desolventizing and toasting the meal; and processing the oil (CCC, 2017) As described below:

- ***a. Seed cleaning:*** consists of selecting only the canola seeds from weed seeds, pods, stems or any other material that may come mixed.
- ***b. Seed pre-conditioning and flaking:*** roller mills are used to rupture the cell walls while maintaining the quality of the oil. If the seeds come from a colder climate, they are pre-heated to 35˚C to prevent shattering when rolled. The optimum thickness of the flakes is between 0.3-0.38mm.
- ***c. Seed cooking:*** the flakes are conditioned/cooked by going through a sequence of stack type cookers or steam-heated drums. The objective of this step is to reduce oil viscosity, thermally rupture oil cells that remained from the flaking step, and adulterate hydrolytic enzymes. One cooking cycle takes about 15-20 minutes with temperatures that range from 80˚C to 105˚C. In the beginning of the cooking step, temperatures increase quickly to 80-90˚C to inactivate the enzyme that hydrolyzes the glucosinolates in toxic compounds that affect the quality of both the meal and the oil. The ideal temperature for the process is about 88˚C.
- ***d. Seed pressing:*** a rotating screw shaft press the flakes in a barrel that contains steel bars aligned and spaced to allow the oil to flow. This process removes 50-60% of the oil content and produces a cake in ideal conditions for the solvent extraction step.
- ***e. Solvent extraction:*** consists of using n-hexane to flood the cake bed saturating it through a series of pumps. The marc is the result of this step, which is a hexane-saturated meal that after a fresh solvent wash, contains less than 1% of oil.

f. ***Meal desolventizing and toasting:*** process is when the solvent is removed and recovered from the marc through a desolventizer-toaster, where the solvent is separated from the meal by being heated on steam-heated plates. The toasting consists of the infusion of steam through the meal. During this 50 to 90-minute process there is an increase of 12-18% in the moisture content and the temperatures range about 95-115˚C. Then the blowing of air through the meal cools and dries it to a 12% moisture content. Next, the meal is consistently granulated by a hammer mill. The meal can then be pelleted or stored as a mash.

g. ***Oil refining:*** is the final step to ensure a good shelf-life and stability of the oil. It involves the precipitation of organic compounds combined with water or simply water on the oil to remove phospholipids, free fatty acids, mucilaginous gums, fine meal particles and colour pigments that might be present. The compounds removed from the oil in this process are then aggregated to the meal so more nutritious components are present for use in animal feeds. A filter with natural clay is used to remove the undesired colour compounds still present in the oil. This process is termed bleaching but without the use of chemicals, it is just a physical process. The deodorization removes the compounds that cause unpleasant taste or odour.

This is a general characterization of canola seed processing, but each processing plant might alter the order of these steps, or the temperatures used according to their needs. Newkirk (2011) discussed how different temperatures during the processing of the seeds affect the availability of amino acids in the meal.

2.3.2. Canola meal

Canola meal is recognized as a premium ingredient in dairy feeds for its palatability, low levels of glucosinolates, high-quality of amino acid profile, high level of rumen undegradable protein, and increased milk production when used in dairy rations (Canolamazing, 2013, 2015, 2019; CCC, n. d. d). The canola meal includes both yellow *(Brassica campestris)* and brown-seeded *(B. napus)* varieties of canola and because it contains high amounts of hull, the meal is high in fiber (30%DM) (Bell and Shires, 1982). Although both types are commonly used, the yellow-

seeded *(B. juncea)* canola has lower oil and fiber and higher protein content than the brown-seeded *(B. napus)* (Theodoridou and Yu, 2013). Theodoridou and Yu (2013) discussed their results and concluded that the higher EE values in the brown-seeded meal is connected to the presence of more condensed tannins in this variety than in the yellow-seeded one, which react with other seed components during the heat treatment in a way that it reduces the oil extracted.

Even though different types of canola meals are used for ration formulation, there are differences between them that affect response on animals' production. Considering this aspect, new methods to evaluate ingredients or diets should be considered an asset for dairy ration formulation. Acquiring the feed ingredients from the same supplier does not guarantee that the same quality of product will be received. As Theodoridou and Yu (2013b) discussed, any alterations in temperature may lead to changes in the structure of proteins, which may influence their availability to digestion.

2.4. Chemical analysis

Nutrient evaluation requires chemical evaluations and estimations of nutrient availability and digestibility, requiring hours of analysis in the laboratory, including the use of animals to determine digestibility and availability of nutrients. Yu (2005) discussed that chemical analysis fails to consider the structures of proteins on protein quality, availability, utilization, and digestibility. Later, Refat et al. (2017) added that essential chemical structures can affect characteristics as ruminal degradability and kinetics, digestibility, utilization of feed and its nutritive value.

To meet that need, however, various molecular spectroscopy techniques have gained space on the evaluation of feed components (Yu, 2004, 2006; Doiron, Yu, McKinnon, and Christensen, 2009; Adeysekara et al., 2011; Khan and Yu, 2013; Theodoridou and Yu, 2013b; Refat et al., 2017), and there is a trend to continue applying these technologies in animal nutrition, aiming on obtaining more accurate results of feeds analyses in a quick and reliable manner. Since they are non-destructive, direct, non-invasive, and rapid bioanalytical techniques.

2.4.1. Energy value

"The capacity for performing work" is the common definition for energy, which can be converted into heat and expressed in calories. A food's energy is stored in its chemical components (Eastridge, 2002).

In the 19th century, scientists acknowledged that carbohydrate, fat, and protein were indispensable organic nutrients and amounts of these nutrients started to be considered for feeding standards (Tyrrell, 2005). The next step was to introduce the idea of digestibility and add it to the feeding standards. Around the same time, the concept of energy balance and net energy gained more acceptance which led to the idea of feeding standards based on net energy values of feeds in Europe and in the United States. Around the middle of the 20th century, only few data on net energy for feeds were available due to the required amount of labor to determine the losses of energy through feces, urine, gas, and heat production. As consequence of this limitation, the total digestible nutrient (TDN) system gained supporters because it was determined by the sum of digestible carbohydrate, digestible protein, and 2.25 times the digestible fat content in the feed. This method, however, this method overestimated the net energy content in forages due to the high content of fiber (Tyrrell, 2005).

The total available heat energy of a feedstuff can be obtained through a bomb calorimeter that provides the gross energy (GE). The digestible energy (DE) of a feedstuff is determined by subtracting the energy excreted in the feces. The amount of energy lost with urine or gas production by the animal, is also not available, so the subtraction of this amount from the DE results is the metabolizable energy (ME). The ruminal fermentation causes losses of energy through heat, called the heat increment (HE). The subtraction of HE from the ME results in the net energy (NE). Consequently, the NE unit can be divided into maintenance (NE_m), growth (NE_g), and lactation (NE_L). This division is required because energy is used for different processes at different efficiencies rates (i.e., energy used for growth is about 50 to 70% as efficient as energy used for maintenance) (Eastridge, 2002).

The NRC 2001 model is divided in two components: supply of nutrients and prediction of energy requirements. Each component is further divided in sub models for: young calves, maintenance, growth, pregnancy, lactation, minerals, vitamins, dry matter intake, amino acids,

reserves, protein and energy supply, and diet evaluation. The model assesses for categories of animals: young calf, replacement heifer, lactating cow, and dry cow (NRC, 2001).

The NRC (2001) model uses mathematical equations to estimate the energy requirements and nutrient supply to dairy cows. It brings a new approach to TDN values where it is now based on the total ration instead of individual ingredients. The NRC 2001 also recognizes that intake effects are caused by the whole diet, not only by certain ingredients, and that these effects are not linear (Tyrrell, 2005).

2.4.2. Cornell Net Carbohydrate and Protein System

Diet formulations have evolved over the years. For many years, supplements were added to diets based on prediction equations that attempted to determine the real nutrient availability and requirements of the animals plus extra nutrients to guarantee the animals would have enough available for production (Fox et al., 2004). This model of diet formulation has led to an increase in costs and an oversupply of nutrients to the animals, which consequently resulted in large amounts of nutrients being excreted and effects on air, soil, and water quality (Fox et al., 2004; Lanzas, Tedeschi, Seo, and Fox, 2007).

To precisely predict nutrient supply and requirements for each class of animals in a production system, and to evaluate rapidly and correctly the suitability of a diet, a better model was developed (Tylutki et al., 2008). The Cornell Net Carbohydrate and Protein System (CNCPS) was developed to precisely predict nutrient supply and requirements for each class of animals in a production system, and to evaluate rapidly and correctly the suitability of a diet generated based on the basics of animal physiology, ruminal function, microbial growth, feed passage and digestion (Fox et al., 2004). It consists of a mathematical model used to assess diet and animal efficiency.

The CNCPS was first released in 1992, in four papers (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993), but since it was first published it has been revised and updated (Fox et al., 2004; Van Amburgh, Foskolos, Collao-Saenz, Higgs, and Ross, 2013). The current version of the model is CNCPS 6.5, the software can be downloaded at (http://blogs.cornell.edu/cncps/purchase/) where the license can also be purchased.

The CNCPS model partitions the carbohydrates and protein of the feed into fractions and classifies them according to their rates of passage and digestion (Lanzas et al., 2007).

In the earliest versions, the carbohydrates were fractioned into four groups: CA (rapidly fermented: organic acids, sugars, and oligosaccharides), CB1 (slower Kd (rate of digestion) than A: soluble fiber and starch), CB2 (available NDF), and CC (indigestible fraction). There were many issues with this classification, including the different extent and rate of fermentation of non-fiber carbohydrates (NFC). The CNCPSv.6.1 divided the carbohydrates into eight fractions: CA1 (acetic, propionic, and butyric acids), CA2 (lactic acids), CA3 (organic aids), CA4 (sugars), CB1 (starch), CB2 (soluble fiber), CB3 (available NDF), and CC (unavailable NDF) (Table 2.1). This broader fractioning of the carbohydrates provides a more appropriate and biologically correct classification according to the reality of the rumen fermentation dynamics (Pan, Yang, Xin, and Xong, 2016).

Protein fractions have also been modified since the first version of the CNCPS. The original version had the proteins divided in five fractions: PA (NPN), PB1 (soluble true protein rapidly degraded), PB2 (partly degraded protein), PB3 (slowly degraded protein), and PC (insoluble in acid detergent, unavailable protein). However, there were some limitations with this classification. For example, the belief that all the NPN entered the ammonia pool and did not provide amino N to stimulate microbial protein production, and that the slowly degraded and unavailable fraction could not be applied to all feeds (Pan et al., 2016). Consequently, based on more recent studies, the CNCPSv.6.5 brought a more accurate fractioning of the proteins, which is now partitioned into: PA1 (ammonia), PA2 (soluble non-ammonia crude protein, soluble true protein), PB1 (insoluble true protein, moderately degradable), PB2 (fiber-bound protein, slowly degraded), and PC (indigestible, unavailable protein) (Chrenková et al., 2014; Li, Zhang and Yu, 2016; Pan et al., 2016; Zhang and Yu, 2012) (Table 2.2).

Table 2.1. Comparison of the carbohydrate fractions in different versions of the CNCPS

CNCPS prior to 6.1		CNCPS 6.1	
CA	Sugars, organic acids, and short oligosaccharides	CA1	Acetate, propionate, butyrate
		CA2	Lactate
		CA3	Organic acids
		CA4	Sugars
CB1	Starch and soluble fiber	CB1	Starch
CB2	Available NDF	CB2	Soluble fiber
		CB3	Available NDF
CC	Unavailable NDF	CC	Unavailable NDF

Pan et al., 2016

Structural and chemical compositional differences in carbohydrates and protein fractions affect their rates of digestion (Kd) and passage (Kp). In ruminant animals, protein and carbohydrates are first degraded in the rumen by the microbiota (i.e., aiming microbial protein synthesis), and the residual parts not degraded in the rumen, continue to the other sections of the gastrointestinal tract where they may be further digested (Pan et al., 2016).

CNCPS contains a feed library with data that is not easily available, consisting of fatty acids and amino acids profiles, digestion rates (Kd), and intestinal digestibility of about 800 feed ingredients that include concentrates, forages, minerals, vitamins, and some commercial products (Higgs, Chase, Ross, and Van Amburgh, 2015). However, all these data contained inconsistencies due to differences in chemical analyses or lack of studies to support the information (Van Amburgh et al., 2013).

Table 2.2. Comparison of the protein fractions in different versions of the CNCPS

CNCPS prior to v.6.5		CNCPS 6.5	
PA	NPN (ammonia, peptides, and amino acids)	PA1	Ammonia
		PA2	Soluble true protein
PB1	Soluble true protein (rapidly degraded)	PB1	Moderately degraded protein
PB2	Intermediately degraded protein	PB2	Slowly degradable protein, bound in NDF
PB3	Slowly degraded true protein		
PC	ADIP (acid detergent insoluble protein)	PC	Unavailable CP

Pan et al., 2016

Since the first version of CNCPS was published, ingredients in the feed library have been altered and added based on reliable sources, such as the National Research Council (Higgs et al., 2015). Recent updates to the library also modified the protein fractions (Van Amburgh et al., 2013).

Physiological state, breed, urea excretion, activity, and environmental effects are considered as part of the equations to predict maintenance requirements (Fox et al., 2004; Ying, 2015). Maintenance (NEm) requirements in growing cattle are calculated based on changes in the body condition scale (BCS) (Fox et al., 2004; Van Amburgh et al., 2015). NEm requirements are regulated according to the animals' activity and energy used to control normal body temperature, which is calculated based on environmental temperature, relative humidity, condition and depth of hair coat, and BCS (Fox et al., 2004).

The CNCPS model predicts body reserves with calculations based on BCS instead of BW, because most producers monitor BCS but not BW, and because there are exchanges in body fat and water balance during lactation (Fox et al., 2004). However, body weight (BW), BW gain, chemical composition of the gain, and mature weight are all included in the calculations to predict protein and energy requirements for growth (Fox et al., 2004; Tylutki et al., 2008).

Pregnancy requirements in the CNCPS are based on the expected calf birth weight, day of gestation, and shrunk body weight (SBW) gain from the growth of the uterus (Tylutki et al., 2008) (Figure 2.1). Lactation protein and energy requirements are based on actual milk components and production (Fox et al., 2004; Tylutki et al., 2008). CNCPS's equations are also capable of predicting dry matter intake (DMI) when it is not known and can be used to compare previously measured intakes (Fox et al., 2004).

The CNCPS model is divided in two levels of prediction of energy and protein supply based on the availability of data to the producer at their farm and the level of confidence of the user. Level 1 should be used when the user has neither the knowledge nor the confidence to use the level 2 (rumen model), and the feeds are not appropriately characterized. The level 2 model is designed for users with appropriate information on dry matter intake (DMI), feed composition, and can understand the use of this rumen model (Fox et al., 2004).

The CNCPS model has many functions, as a teaching tool, a diet formulating program, a tool in planning nutrient management, etc. The use of CNCPS has allowed a reduction of 1-2% in the total crude protein in the diets which lowered the costs and without compromising performance (Fox et al., 2004). It has been proved to be precise on rumen fermentation characteristics, feed chemistry, and more biologically correct, and has been effectively used to reduce the impacts on the environment from ruminant production systems and the costs of feeds (Ying, 2015).

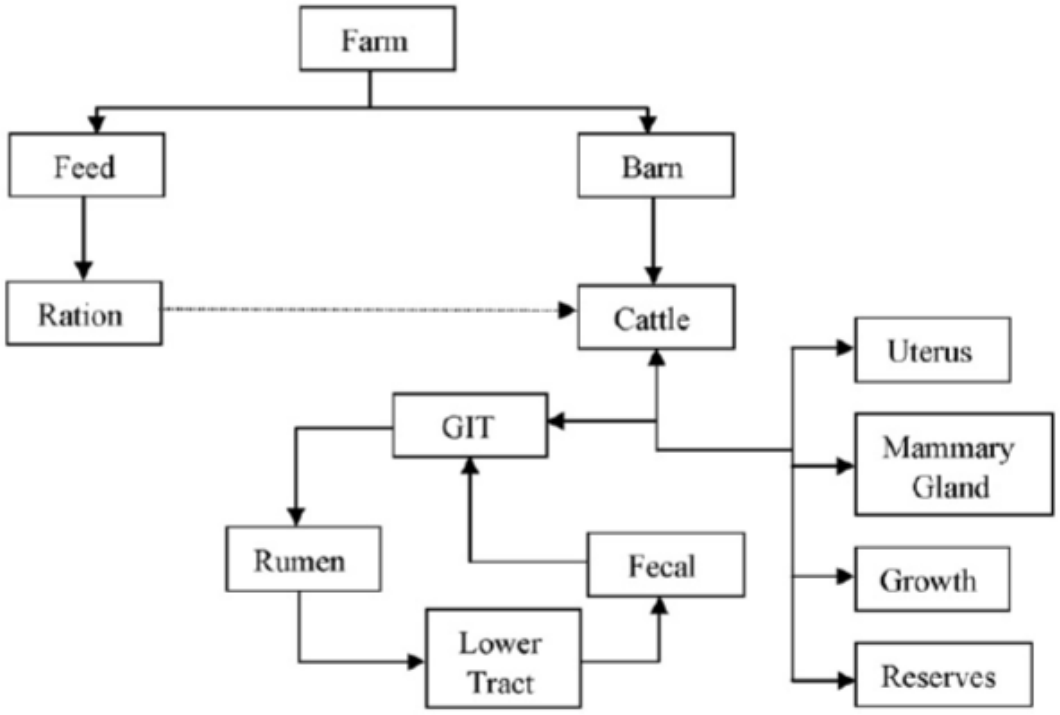

Figure 2.1. Diagram of the object-oriented programming structure of the Cornell Net Carbohydrate and Protein System. (Tylutki et al., 2008).

*2.4.3. **In situ** technique to determine rumen degradation kinetics*

It is highly recognized how important the knowledge on ruminal protein degradation is, and it has been the main subject in various studies over the years (Ørskov and McDonald, 1979). The nylon bag *(in situ)* technique gained appreciation for being an inexpensive and reliable method to obtain relevant ruminal physiological knowledge and chemical composition of feedstuffs. To apply the technique, fistulated animals and nylon bags are required. Once the animals are acquired and surgically fistulated, they can be used to help science for many years without health problems.

Scientist have preferred to use *in situ* techniques to characterize feedstuffs, using artificial fiber bags in the rumen, they have had quick access to the rate and extent of degradation of feedstuffs just by weighing the bags (Ørskov, Hovell, and Mould, 1980). The nylon bag incubation

method has become universally accepted and used to estimate the contribution of CP (Tamminga et al., 1994).

Although extensively applied, the nylon bag method underestimates the rate of degradation of cell walls, indicating that ruminal CP degradation is more dynamic than can be simulated with the nylon bags (Tamminga et al., 1994).

Without problems, scientists may adapt the technique to the reality of their samples. For instance, to assess the degradation of organic matter, dry matter, CP, NDF, and starch, in corn and wheat distillers dried grains, Damiran et al. (2013) applied the nylon bag method using samples of 7g in pre-weighed 10×20cm nylon bags with pores size of 40 µm. The samples were incubated in the rumen in a "gradual addition/all out" schedule for 0, 2, 4, 8, 12, 36, and 72 hours. The 0 h samples are not incubated in the rumen, but they are washed the same way as the incubated bags. After the removal of the bags from the rumen when the incubation period is over, the bags are rinsed in cold water until clear water runs and are then dried at 55˚C for 48h, and then they can be either analyzed or stored for future analysis. Heendeniya, Christensen, Maenz, McKinnon, and Yu (2012) when analyzing canola meal, applied the same method with the same bag type to obtain values for OM, DM, CP, NDF, and ADF, for the following incubation times 0, 2, 4, 8, 12, 24, 48, and 72 h. And Yu, Goelema, and Tamminga (2000) analyzing horse beans applied the nylon bag method using 5.5g samples in 10×17cm bags with 40 µm pore size for 0, 2, 4, 8, 12, and 24 h when analyzing horse beans for DM, N, starch, and ash.

Mathematical equations are used to describe the kinetics of rumen degradation of CP, DM, NDF, and starch. The equations consider the duration of incubation (*t*), the undegradable (U) and the potentially degradable fractions (D), the lag time (T0) and the rate of degradation (Kd) (Damiran et al., 2013).

$R(\mathrm{t}) = U + D \times \exp\left(-Kd \times (\mathrm{t} - T0)\right)$ for OM, NDF, and CP

$R(t) = D \times \exp(-Kd \times t)$ for starch

Other equations are used for effective degradability (ED), rumen undegraded feed protein (RUP), and rumen undegraded feed starch (RUST).

This nylon bag method is an important tool for science because for ruminants, the availability of amino acids that leave the rumen is more relevant than the content in the diet, due to the extensive microbial activity that takes place in the rumen (Hvelplund, Weisbjerg, and Andersen, 1992).

*2.4.4. Determining intestinal digestion using a three-step **in vitro** technique*

Synthesis of milk and tissues rely on the availability of amino acids. Ruminal microbial protein presents consistent digestibility and profile, being the main source of absorbable amino acids that flow to the small intestine. The second source of amino acids corresponds to the rumen undegraded protein, however the digestibility of rumen undegraded protein is variable and depends on feed type and processing (Gargallo, Calsamiglia, and Ferret, 2006).

The protein available for absorption in the small intestine depends on the flow of dietary and microbial N and their digestibility. The contribution from intestinal digestion in ruminants becomes more important when there are high levels of undegraded intake protein in the diet (Calsamiglia and Stern, 1995).

Before the development of an in vitro method, the estimation protein digestion in the small intestine required surgically adapted animals, long hours of intense labour, and was expensive. The *in vitro* method created by Calsamiglia and Stern in 1995 was developed to achieve four goals: to mimic real physiological conditions; to be rapid, reliable, and low cost; to be reproduced to a variety of protein sources; and, to precisely demonstrate the differences in protein digestion (Calsamiglia and Stern, 1995).

This *in vitro* method is defined as a three-step procedure (TSP) because it considers the ruminal, gastric, and pancreatic actions on the feed, in order to determine the digestibility of the protein in that feed. The ruminal digestibility, according to the TSP, is determined after a 16h incubation of the feed sample in the rumen of a fistulated animal. The residue from this step is then used in the pepsin and pancreatin digestion, to determine the intestinal digestion of the protein, and consequently estimate the total tract digestion of the protein in that feed. This technique has

been shown to be sensitive to the presence of anti-trypsin factors and to heat damage on the feed (Calsamiglia and Stern, 1995).

Although the TSP has been proved efficient and more affordable when compared to the regular animal trials to determine protein digestibility, it requires an expensive enzyme and uses a very corrosive and toxic chemical (trichloroacetic acid [TCA]) in one step of the procedure (Gargallo et al., 2006). Attempting to minimize these problems, Gargallo et al. (2006) slightly modified the original three-step procedure. They used a Daisy^{II} incubator with a less expensive enzyme, and reduced the rumen incubation time to 12h, and the need to change bags for the *in vitro* incubation after the rumen incubation. Results are statistically equal to the original method (Gargallo et al., 2006; Wang et al., 2015).

Wang et al. (2015) compared the mobile nylon bag method ([MNB] using cannulated animals in various segments of the gastrointestinal tract), the modified three-step *in vitro* method, the TSP, and the acid detergent insoluble nitrogen method (ADIN). They concluded that the mobile nylon bag method can be replaced by either the TPS or the modified TPS to determine the digestibility of rumen undegraded protein from roughages and concentrates, but the values obtained with the modified TPS were closer to the values obtained through the MNB method when concentrates were analyzed, proving that these methods are reliable and can be used instead of methods that require animals.

2.4.5. Prediction of truly absorbed protein supply to the small intestine

a. NRC-2001 system

The NRC-2001 model defines the true protein that is digested and absorbed in the intestine as metabolizable protein (MP). The NRC considers the feed protein undegraded in the rumen; the microbial protein synthesized in the rumen; and the endogenous protein that comes from the rumen as potentially contributing to MP. The rumen degraded protein balance (DPB), in the NRC model, is calculated by the possible microbial protein that is synthesized in the rumen, depending on the feed CP degraded and the energy available for fermentation (total digestible nutrients [TDN]) in the rumen (NRC, 2001; Yu, Meier, Christensen, Rossnagel, and McKinnon, 2003; Yu, 2005).

b. Dutch DVE/OEB system

The DVE/OEB Dutch system is a modern protein evaluation method (Tamminga et al., 1994). It considers the truly absorbed protein and the degraded protein in the small intestine. In this system, both the requirements for dairy cows and the protein value of feeds are expressed in amounts of protein (feed and microbial source) that is truly absorbed and digested in the small intestine of the cow. The DVE/OEB system gives a DVE value to each feed based on three factors: true digestible protein that escapes the rumen degradation (ABCP); true digestible microbial protein (AMP) that was synthesized in the rumen; and a correction for the endogenous losses of protein (ENDP) in the tract. Plus, it also gives an OEB (rumen degraded protein balance) value that represents the balance between the possible microbial protein synthesized from the energy extracted during the fermentation in the rumen (E_MP) and the available degradable protein in the rumen (N_MP) (Yu, Goelema, Leury, Tamminga, and Egan, 2002; Yu, 2005; Nuez-Ortin and Yu, 2010). If the OEB value is positive, it means there is potential to the loss of N of the feed from the rumen; whereas, if it is negative, there might be a shortage of N that is risk to the microbial protein synthesis (Tamminga et al., 1994).

2.4.6. Feed Milk Value

The net energy value of a feed for lactation is measured by the increase in milk energy yield that is associated with the increased intake of the feed, considering that all the other aspects remain unchanged during the increase of the intake and the measurement of the milk yield (Tyrrell, 2005).

Dietary CP is positively correlated with milk yield not with milk protein yield. Milk protein production can be elevated when the amino acids profile in the metabolizable protein is improved, the excess of protein is reduced in the diet, and when the volume of fermentable carbohydrate of the diet in increased (NRC, 2001).

The feed milk value (FMV) can be determined according to the metabolic characteristics from the DVE/OEB and NRC models (Rodriguez, 2018).

2.5. Mid-infrared vibrational spectroscopy

Zhang and Yu (2014) showed that the profiles of the secondary structure of proteins can impact protein nutritive use and functionality, due to their influence on the accessibility to enzymes in the gastrointestinal tract. Also, Abeysekara, Damiran and Yu (2011) and Khan and Yu (2013) highlighted that to properly understand the nutritive quality, digestive behavior, availability, and utilization of proteins in animals, the molecular structures of the protein, such as α-helix and β-sheet, should be considered. They also pointed that α-helix and β-sheet ratio affected both the degraded protein balance (OEB value) and the total absorbed protein supply (DVE value) in the intestine (Abeysekara et al., 2011; Khan and Yu, 2013).

2.5.1. Fourier Transform Infrared Radiation (FTIR)

The Fourier-transform infrared spectroscopy technique (FTIR) is a method that uses different wavelengths of infrared light to explore the macromolecules (e.g., related proteins, CHO, lipids) in biological samples based on the specific frequency of absorption by each molecule, without adding unintentional perturbations (Stavitski et al., 2013; Gelfand, Smith, Stavitski, Borchelt, and Miller, 2015; Oinas et al., 2016). This technique can provide useful data on the composition of individual components and on the arrangements of proteins at chosen locations of a sample (Ling, Qi, Shao and Chen, 2015). Because of its high sensitivity FTIR has also been successfully used to determine modifications on the secondary structures of proteins (Gelfand et al., 2015).

FTIR microspectroscopy (FTIR-MS) is defined as the combination of microscopy with the conventional spectroscopy which favors the exploration of the molecular chemistry in small samples at a microscopic level, generating chemical imaging (Wang, Yao and Parthasarathy, 2008; Oinas et al., 2016). Each pixel of a FTIR image is formed by an absorption spectrum that when

explored shows the structures and the spatial distribution of the various biochemical components present in a section of the sample (Oinas et al., 2016).

This technique has been in chemistry, biology, and other fields to obtain data on the microstructures of different materials, however the development of analytical techniques to obtain FTIR images has made FTIR even more efficient because prior to the analytical techniques, the microscope mapping of a sample took several days (Wang, Yao and Parthasarathy, 2008). With these new analytical methods, tissue chemistry, structure, and composition data can be displayed at the same time.

2.5.2. Synchrotron

A synchrotron is an enormous particle accelerator that creates light from the acceleration of electrons (Yu, 2004) and the light produced is used to visualize the molecular chemical structures of materials (Canadian Light Source [CLS], n.d.). The synchrotron is composed of 6 components which are: an electron gun, a linear accelerator, a booster ring, a storage ring, beamlines and end experimental stations (Yu, 2006). The light produced by a synchrotron is millions of times brighter than sunlight (Yu, 2004).

The synchrotron uses potent electromagnets and radio frequency waves to produce light. As the electrons accelerate, more energy is added to the electrons, and when the course of an electron beam is modified by the powerful magnets in the system, the electrons radiate a bright and focused light. Various light spectra, such as ultraviolet, x-rays and infrared, are conducted to the beamlines, where the scientists choose which wavelength is the most adequate to study their specimens at the end stations. A synchrotron is used to explore materials and to study their chemical, physical, geological, and biological characteristics. This technology is applied in the design of new drugs, in building smaller computer chips, development of new materials to use in medicine, and various other ends (CLS, n.d.).

The combination of microscopy, FTIR spectroscopy and a synchrotron light source originated the synchroton radiation-based FTIR microspectroscopy (S-FTIR) (Wetzel, Eilert, Pietrzak, Miller, and Sweat, 1998; Yu, 2004, 2006). The S-FTIR produces a bright light from the

acceleration of electrons integrated with microscopic imaging, which from the spectra generated at a molecular level, provides scientists with information regarding the functional groups, structures, spatial distribution, and quantity of chemical constituents present in the sample (Stavitski et al., 2013; Yu, 2004, 2006).

2.5.3. Attenuate Total Reflectance Fourier-transform Infrared Vibration Spectroscopy (ATR-FT/IR)

Another spectroscopy method that has been developed as a nondestructive, noninvasive, direct, and fast bioanalytical technique to obtain the physiochemical characteristics of feed is called Attenuate Total Reflectance Fourier-transform Infrared Vibration Spectroscopy (ATR-FT/IR) (Refat et al., 2017). Theodoridou and Yu (2013b) described the ATR-FT/IR as an advanced well-stablished technique used for the study of structural stability, composition, and conformational changes on a molecular basis due to the effects of pressure, pH and temperature. Refat et al. (2017) also commented that this technique can be applied to quantify the content, structures, composition, distribution of functional groups and chemical constituents of a sample with various chemometrics.

2.5.4. Spectral Analysis Methods

Data obtained through the various spectroscopy techniques need to be treated by some spectral analysis method to be useful.

a. Univariate

Univariate spectral analysis is the foundation to analyze the data collected through FTIR-MS (Oinas et al., 2016). This analysis method produces functional group images based on band areas, intensities, and ratios, and each of these images are formed by the frequency as function of the intensity of the spectra and its spatial position (Wang, Yao and Parthasarathy, 2008). The FTIR

images, or maps, generated in this univariate mode, displays the specific contrast related to the chemical bonds in the specimen analyzed.

Univariate spectral analysis methods are easy to use but they do not take into consideration a large amount of spectral information and therefore may not provide the most accurate information (Oinas et al., 2016). These methods allow the reading of information about the relative concentration and the distribution of a specific functional group; however, they are not very efficient at displaying the classification of histopathological and chemical characteristics in a sample, which makes it difficult to detect minor differences in the spectra (changes in the chemical composition along the area) (Wang et al., 2008).

The univariate technique forms images based on the difference in the absorption peaks by the units in the sample. When applying this technique, it may not be able to represent the differences in proteins, because the characteristic peaks of proteins are very close to one another making it challenging to distinguish their differences (Ling et al., 2015).

b. Multivariate

Multivariate spectral analysis methods use data from the full spectrum, which generates more accurate and detailed information about the sample (Oinas et al., 2016). These techniques analyze the data collected not by considering individual bands in a spectrum, but by considering each pixel (spectrum) (Wang et al., 2008).

There are several multivariate analysis methods, such as hierarchical cluster analysis, fuzzy c-means clustering, k-means clustering, and principal components analysis (PCA), All these methods classify spectra according to their similarity, and are later used to differentiate chemical patterns in the microstructures of a sample (Wang et al., 2008).

Principal components analysis (PCA) is a statistical method that reduces the data by converting the initial series variables into a new series of uncorrelated variables, named PCs (Theodoridou and Yu, 2013b).

Principal components analysis (PCA) imaging permits the reduction of variables because it creates a linear combination of principal components (PCs), which are wavenumbers that vary together. The first PC mostly clarifies all the data variance. While the second PC explains most of the remaining variance. The contribution of each PC to the spectrum is determined by each of their projection on the spectrum (Ling et al., 2015). PCA is a statistical method that reduces the data by converting the initial series variables into a new series of uncorrelated variables, named PCs (Theodoridou and Yu, 2013b).

Cluster analysis (CLA) is one of the multivariate analysis methods. It arranges the spectra in groups, and its use minimizes the difference inside each group and maximizes the differences between other groups. It is used to partition FTIR images (Oinas et al., 2016).

2.6. Literature Review Summary, Hypotheses and Objectives

2.6.1. Literature review summary

Over the years many spectroscopy techniques have been developed to study the small constituents of materials and cells. To advance the use of these technologies, statistical analytical methods have been used as part of the process of acquiring reliable and useful data. These techniques have been applied to chemistry, biology, electronics industries etc. However, few studies have used them in feed science. Along with previous information on availability and digestibility of nutrients, spectroscopy can be a tool in the prediction of nutrient utilization and animal production. To simplify the future of chemical analyses, diet formulation and animal production, it is necessary that studies applying molecular spectroscopy on feed ingredients are held to obtain more background information on the protein and carbohydrate structures that are crucial to determine the availability and digestibility of nutrients to ruminants.

2.6.2. Hypotheses

- There are significant variations in molecular structure features and nutritional profiles among bio-oil processing plants and between countries.
- There is a high association between the molecular structure features and nutrient utilization and availability of feedstocks and co-products regardless of the origin of the product.

2.6.3. Objectives

General research objective:

- To reveal association of intrinsic molecular structure with nutrient supply to animals from feedstocks and co-products from bio-oil processing using advanced vibrational molecular spectroscopy techniques

Detailed research objectives:

- To characterize the chemical and nutrient profiles of feedstock (oil seed) and co-products (meal or pellet) from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To determine digestible, metabolizable and net energy values of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To determine protein and carbohydrate subfractions using newly updated CNCPS 6.5 system of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To determine degradation and digestion of each protein and carbohydrate fractions in the rumen with CNCPS6.5 system of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To understand rumen fermentation/degradation and intestinal digestion in dairy cattle of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries

- To utilize molecular spectroscopy to characterize the molecular structures of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To quantify the relationship between molecular structure and nutrient utilization and availability of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries
- To predict nutrient supply and rumen and intestinal digestion using molecular spectral features of feedstock and co-products from bio-oil processing: Comparison among bio-oil processing plants and between two different countries

3. CHARACTERIZATION OF CHEMICAL AND NUTRIENT PROFILES OF FEEDSTOCKS (CANOLA SEEDS) AND CO-PRODUCTS (CANOLA MEALS AND PELLETS) FROM BIO-OIL PROCESSING: COMPARISON BETWEEN CANADA AND CHINA

3.1. Abstract

Since its development in 1974, canola has been largely produced specially in Western Canada. Canola is an oilseed that is mainly produced for the extraction of its oil for human consumption. The extraction process generates a co-product, canola meal, that is low in glucosinolates and high in good quality protein, which makes this product ideal for animal supplementation. Variations in crop conditions and seed processing can affect the quality of seeds and meals produced. This study aimed on characterizing and comparing canola seeds and meals (mash and pellet) from different bio-oil processing plants in Canada and in China collected in 2016 by the Canola Council of Canada, regarding the chemical composition, energy profile (NRC 2001) and protein and carbohydrate fractions (CNCPS 6.5). DM was higher on Canadian canola meals (89.96 vs. 88.55%, $P<0.001$). CP was higher in Chinese meals (43.04 vs. 41.87% DM, $P=0.003$). EE was not different between countries ($P>0.05$). However, on canola seeds, only DM was higher in Canada (93.10 vs. 92.28%, $P=0.008$), while CP and EE were similar between countries ($P>0.05$). TDN_{1x} was similar between canola meals regardless of the country ($P>0.05$). Chinese meals and seeds had higher tdCP and tdNDF than Canada's ($P<0.05$), while Canada had higher tdNFC ($P<0.05$). ME_{3x}, NE_{Lp3x}, $NE_{m3x,}$ NE_{g3x} were similar in canola meals from both countries ($P>0.05$). No differences were observed between the energy profile of canola seeds from either country ($P>0.05$). The protein and carbohydrate fractions of canola seeds within China were similar ($P>0.05$). Contrast analysis showed that pelleting affected the protein fractionation of Canadian canola meals

(P<0.05), except for PB1, RDPB1, RUPB1, and DIGPB1. Canola meals were different between Canada and China on the soluble (PA2) and slowly

degradable fractions (PB2) ($P<0.001$). CB2, CB3, and CC were different among Chinese meals ($P<0.05$). China presented higher CA4 ($P=0.04$) and lower CB2 ($P=0.01$), and CC ($P<0.001$) than Canadian canola meals. Although the seeds were similar within and between counties, the oil-extraction process and pelleting seemed to have generated some different aspects on the meals in both countries, however the high quality is still comparable.

3.2. Introduction

Canola has been produced in Western Canada since 1974, when it was developed as a low erucic acid and low glucosinolate rapeseed, to supply for the high demand of cooking oil (Eskin, 2016). When canola oil is extracted, it generates a co-product low in fat and rich in protein. This co-product, canola meal, is mainly used in dairy rations because its amino acid profile is ideal for milk synthesis (Maesoomi, Ghorbani, Alikhani, and Nikkhah, 2006).

Due to the high production of canola and the high global demand, besides being extensively used in Canada, it is also exported to many countries. China is one of the main markets for Canadian canola seeds and its product and co-product (Canola Council of Canada (CCC),2019).

Different crops and seed processing methods can alter the composition of the nutrients (Newkirk, 2011) and the protein profile of canola meals. Meaning that canola meals should not be assumed equal before prior to proper testing.

Canola meal is a co-product that contains outstanding rumen degradable (RDP) and undegradable protein (RUP) profiles that stimulates both microbial growth and milk synthesis (Piepenbrink and Schingoethe, 1998). White et al. (2017) defended the prediction of rumen undegradable protein (RUP) because of its importance for dairy rations, as RUP content can impact both the microbial protein synthesis and the amino acid profile that will be available for absorption in the small intestine of the animal.

The aim of this study was to characterize the chemical composition and nutrient profiles of canola seeds and meals from five different seed crushing plants in Canada and five different seed

crushing plants in China, using standard wet laboratory analyses, and the NRC 2001 and CNCPS 6.5 models.

3.3. Materials and Methods

3.3.1. Sampling

The samples of feedstocks and co-products from bio-oil processing were collected from Canada and China by the Canola Council of Canada in 2016. The samples were provided by each company's quality control laboratory and are to be considered representative of the reality of those crushers.

Samples were collected from five crusher companies operating in four provinces in China. These companies only crushed seeds imported from Canada. Samples of seeds and meals were collected from different batches from each crusher, stored and transported to the University of Saskatchewan in Canada for further analyses.

Samples of seeds and meals were also collected from five crushers in Canada. However, three of the five Canadian crushers samples of meals were pelleted and two were mash, unlike China's meals that were all mash. Samples were collected from different batches from each crusher, stored and transported to the University of Saskatchewan for future analyses.

All samples of seeds were ground using a blade coffee grinder, model BCG111OB manufactured by KitchenAid®, USA. The samples of meals that were pelleted were ground using a 1mm screen on the grinding mill, Ultra Centrifugal Mill ZM200 manufactured by Retsch®, Germany.

3.3.2. Chemical Analysis

The chemical analysis of the samples followed the analytical procedures described on the Official Methods of Analysis 21st Edition (2019) for Dry matter (DM), ash, crude protein (CP),

crude fat (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose, cellulose, non-fiber carbohydrate (NFC), non-structural carbohydrate (NSC). For neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP), the procedures by Licitra, Hernandes, and Van Soest (1996) were followed. To determine the soluble CP (SCP) content of the samples, the methodology by Roe, Sniffen, and Chase (1990) was applied.

3.3.3. Energy profile

The energy values and digestible nutrient profiles of the feedstocks and co-products from bio-oil processing were determined based on the chemical profile, according to the National Research Council (NRC): Nutrient Requirements for Dairy Cattle (2001).

$$\boldsymbol{tdFA} = FA\ or\ if\ EE < 1, FA = 0$$

$$\boldsymbol{tdNDF} = (0.75 \times ((NDF - NDICP) - ADL \times (1 - (ADL \div (NDF - NDICP)^{0.667})$$

$$\boldsymbol{tdCP} = (1 - \left(0.4 \times (ADICP \div CP)\right) \times CP$$

$$\boldsymbol{tdNFC} = 0.98 \left(100 - \left((NDF - NDICP) + CP + EE + Ash\right)\right) \times PAF,$$ where PAF = 1

$$\boldsymbol{TDN_{1X}} = tdNFC + tdCP + (tdFA \times 2.5) + tdNDF - 7$$

$$\boldsymbol{DE_{1X}} = ((tdNFC \div 100) \times 4.2 + \left((tdNDF \div 100) \times 4.2\right) + \left((tdCP \div 100) \times 5.6\right) + \left((tdFA \div 100) \times 9.4\right) - 0.03$$

$$\boldsymbol{ME} = 0.82 \times DE_{1X}$$

$$\boldsymbol{NE_g} = (1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65)$$

$$\boldsymbol{NE_m} = (1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12)$$

If EE<3%, $\boldsymbol{NEL_P} = (0.703 \times ME - 0.19)$

If EE≥3%, $\boldsymbol{NEL_P} = (0.703 \times ME - 0.19) + (((\left((0.97 \times ME) + 0.19\right) \div 97 \times (EE - 3))$

3.3.4. CNCPS 6.5 System

The Cornell Net Carbohydrate and Protein System (CNCPS) partitions carbohydrates and proteins into fractions based on rates of passage and digestion. Carbohydrates were fractionated into volatile fatty acids (CA1), lactic acid (CA2), other organic acids (CA3), water soluble carbohydrate (CA4), soluble fiber (CB2), digestible fiber (CB3), indigestible fiber (CC). The protein fractions correspond to ammonia (PA1), soluble true protein (PA2), insoluble or moderately digestible true protein (PB1), fiber-bound or slowly digestible protein (PB2), and unavailable or indigestible protein (PC) (Higgs et al., 2015).

Following the fractionation of proteins and carbohydrates, the ruminal degradability and intestinal digestibility were also predicted based on the model.

$$\boldsymbol{CHO} = 100 - CP - EE - Ash$$

$$\boldsymbol{CC} = \frac{aNDFom \times (Lignin \times aNDFom) \times 2.4}{100}$$

$$\boldsymbol{CB3} = aNDFom - CC$$

$$\boldsymbol{NFC} = CHO - aNDFom$$

$$\boldsymbol{CB2} = NFC - CA1 - CA2 - CA3 - CA4 - CB1$$

$$\boldsymbol{CA1} = Acetic + Propionic + (Butyric + Isobutyric)$$

$$\boldsymbol{CA2} = Lactic$$

$$\boldsymbol{CA3} = Organic\ acids$$

$$\boldsymbol{CA4} = Water\ Soluble\ Carbohydrates$$

$$\boldsymbol{CB1} = Starch$$

$$\boldsymbol{PA1} = Ammoniaj \times \left(\frac{SP}{100}\right) \times \left(\frac{CP}{100}\right)$$

$$\boldsymbol{PA2} = SP \times \frac{CP}{100} - PA1$$

$$\boldsymbol{PB1} = CP - (PA1 - PA2 - PB2 - PC)$$

$$\boldsymbol{PB2} = (NDICP - ADICP) \times \frac{CP}{100}$$

$$\boldsymbol{PC} = ADICP \times \frac{CP}{100}$$

3.3.5. Statistical Analysis

To better accommodate the variations and prevent statistical errors, the statistical design of this study is a Complete Randomized Block Design (RCBD), where country and company are fixed effects and batch is a random effect. The procedure MIXED was used on SAS® 9.4 (SAS Institute, USA).

$$y = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

Where, μ= overall mean; τ_i= fixed effect; β = random effect; ϵ_{ij}= error.

β_j ~ NIID (Normally, Identically, and Independently distributed)

ϵ_{ij} ~ NIID (Normally, Identically, and Independently distributed)

Significance was declared at $P<0.05$. The Tukey method was used for the multiple comparison test.

3.4. Results and Discussion

3.4.1. Chemical profiles of feedstocks and co-products: comparison among bio-oil processing plants and between two countries

The chemical profile of canola meals is represented in Table 3.1 and Table 3.2. On this study, canola meals dry matter averaged higher on the samples collected in Canada (89.96%) than on the samples from China (88.55%) ($P<0.001$). Crude protein (CP) was lower for the Canadian samples (41.87%DM vs. 43.04%DM (or 37.7 and 38.1% by mass) ($P=0.003$)). Ether extract (EE) was not different between Canadian and Chinese samples (0.79%DM vs. 0.47%DM (or 0.7 and 0.4% by mass) ($P=0.118$). Acid Detergent Fiber (ADF) was also similar between countries (20.86%DM (CA) vs. 20.56%DM (CH) (or 18.8 and 18.2% by mass) ($P=0.408$).

According to the Canadian Oilseed Processors Association (COPA) (2020), a maximum of 12% moisture and 12% of crude fiber, and a minimum of 36% of protein and 2% of fat (solvent extracted), (measured in % by mass) are the standard specifications for canola meal. The 2020 Canola Annual report (CCC, n.d.) complied data from 7 years with samples from 13 different Canadian plants and found as average chemical composition that canola meals had 42% CP, 3.2% EE, 18.6% ADF (on a DM basis), and 12% moisture.

Paula et al. (2018) reported CP as 41.8%DM, NDF as 28.9% DM, and ADF as 18.6%. On a review, Paula et al. (2019) reported canola meal with 91.4% of DM, 39.8%DM of CP, 19.4%DM of ADF, 28.5%DM of NDF, and 4.56%DM of EE. Mustafa, Christensen, and McKinnon (1997) reported the profile of canola meal as 42%DM of CP, 24%DM of NDF, and 19%DM of ADF. While Broderick and colleagues (2015) reported using canola meal with 89.6% of DM, 40.6%DM of CP, 3.0%DM of EE, 29.9%DM of NDF, 18.2%DM of ADF, 26.2%CP of NDICP, and 6.2%CP of ADICP.

Based on these results, the canola meal samples analyzed for this project were in accordance with these values previously reported, except for the EE which was lower than reported by Paula et al. (2018), Paula et al. (2019), CCC (2021) and expected by COPA (2020). Our EE values for canola meals averaged 0.79%DM for the samples from Canada and 0.47%DM for the

ones from China; however, the samples from plants 3 and 4 from Canada that were pelleted presented EE of 1.46 and 1.06%DM respectively, which can be associated with the coating of the pellets with oil, but this higher EE was not observed on the pellets from plant 5 (0.63%DM).

Soluble crude protein (SCP) and Neutral detergent indigestible crude protein (NDICP or NDIP) were different between Canada and China. While China presented higher CP (43.04% vs. 41.87%DM (P=0.003)) and SCP (22.51% vs. 17.11%CP (P<0.001)), Canada presented higher NDICP (19.34% vs. 13.52%CP (P<0.001)). Acid detergent insoluble crude protein (ADICP) was not significantly different (P=0.075) (Table 3.1).

Mustafa et al. (1997) stated that the NDICP of regular canola meal was 105 g/kg CP which is lower when compared to meals from CA that averaged 19.34%CP, but close to the samples from plants A and C from China (11.07 and 10.83%CP), however, still lower than China's average of 13.52%CP. They also reported ADICP as 45 g/kg CP was lower than this project's meals (CA (5.53%CP) and CH (5.80%CP)).

According to Newkirk (2011) different cultivars, canola growth environments and harvest, and the processes the seeds and meals go through can all affect the final nutrient profile of the meal. Since five different companies were sampled in the production of different batches of meals both in Canada and in China, it is safe to assume that these results are representative of the companies and their quality is steady through different batches, and small variations are expected due to the variability of crop conditions, cultivars, and harvest.

The chemical profile of the canola seeds studied on this project is displayed in Table 3.3 and Table 3.4. The DM of seeds from Canadian plants was higher (93.1%) than those from Chinese plants (92.28%) (P=0.008). CP content was similar (P=0.100) (22.46%DM for CA vs. 22.20%DM for CH). SCP was higher for CH plants (54.30 vs. 48.21%CP (P=0.002)). And NDICP was higher for CA plants (10.63 vs. 9.11%CP (P<0.001)). NDF, ADF and Cellulose were higher for CA plants (P=0.004, P=0.003, and P<0.001, respectively), while ADL was higher for the CH plants (P=0.017).

Park, Ragland, Helmbrecht, Htoo, and Adeola (2019) studied samples of canola seeds, canola meals from solvent extraction and canola meals from expellers. For canola seeds, they reported DM of 94.9%, ash of (3.04%DM), CP of 24.8%DM, NDF of 19.4%DM, and ADF of

15.5%DM. Averaging CA and CH together (considering that the seeds crushed in China were exported from Canada) and comparing to these results, our seeds had higher moisture content (92.7%), higher ash (3.8%DM), lower CP (22.3%DM), lower NDF (16.4%DM), and lower ADF (12.1%DM).

Canola seeds used by Tramontini (2009) were composed of 23.51%DM of CP, 37.34%DM of EE, and 26.52%DM of NDF. Tramontini's seeds were higher in CP and NDF contents (ours were 22.3%DM and 16.4%DM, respectively), and lower in EE (ours averaged 43.3%DM).

The Canadian Grain Commission (CGC) (2021) summarized the canola seed production of 2020 and observed an oil content of 44.1%DM and CP of 20.8%. On their report from the 2015 (CGC, 2016) production, they observed seeds with 44.2% EE and 20.7%DM of CP. These results give us a basis to safely assume that Canada produces canola with a high and stable along the years.

Burbulis and Kott (2005) investigated the variation in color and oil content influenced by the environmental temperature on black-seeded spring rapeseed varieties Brassica napus L. 'Bolero' (owned by Raps GbR) and 'Star' (owned by Dansk Planteforaedling/DLF) and 11 lines originated from their crossing. They found that temperatures higher than 28°C during the day, resulted in offspring with lighter seeds (more yellow) and temperatures lower than 20°C resulted in darker seeds (more brown or black). They also observed differences in oil content on the seeds from different environments. The oil content of the darker seeds (colder climate) ranged from 31.2 to 51.6%DM, and lighter seeds (warmer climate) ranged from 31.4 to 49.4%DM. On average, lighter seeds presented lower oil content.

Tramontini (2009) likely used canola seeds from a different climate, since her study was conducted in Brazil, a tropical country with higher temperatures, as Burbulis and Kott (2005) study suggests, the higher temperatures in that country could have influenced the seeds she used, explaining the lower EE content. The seeds analyzed on our project, however, were in accordance with the standard quality of the Canadian canola seeds.

The higher cellulose content on the CA plants ($P<0.001$) could have been the cause for higher contents of NDF (16.96 vs. 15.87%DM ($P=0.004$), ADF (12.49 vs. 11.77%DM ($P=0.003$)), and NDICP (10.63 vs. 9.11%CP ($P<0.001$)) on the samples from that country.

Table 3.1. Chemical composition profile of co-products from different oil processing plants (canola meal and pellet): comparison among bio-oil processing plants and between Canada and China.

	Basic chemical profile				Protein profile						
Items	DM (%)	Ash (%DM)	EE (%DM)	FA (%DM)	CP (%DM)	SCP (%DM)	SCP (%CP)	NDICP (%DM)	NDICP (%CP)	ADICP (%DM)	ADICP (%CP)
					Canadian processing plants						
Plant 1 (M)	90.28	7.60^{b}	0.68	0.47	42.62^{a}	7.08^{b}	16.63^{b}	7.65^{ab}	17.95^{ab}	2.47^{a}	5.80^{a}
Plant 2 (M)	89.49	8.24^{a}	0.79	0.44	40.94^{b}	7.05^{ab}	17.20^{ab}	8.70^{a}	21.30^{a}	2.45^{ab}	5.98^{a}
Plant 3 (P)	83.13	8.25^{a}	1.46	1.11	41.64^{b}	7.92^{ab}	19.01^{ab}	6.03^{bc}	14.51^{b}	2.02^{c}	4.87^{b}
Plant 4 (P)	89.83	7.43^{b}	1.06	0.74	41.70^{b}	8.57^{a}	20.55^{a}	6.03^{c}	14.48^{b}	2.37^{ab}	5.69^{a}
Plant 5 (P)	89.25	7.28^{b}	0.63	0.28	41.84^{ab}	7.46^{ab}	17.82^{ab}	7.83^{a}	18.73^{ab}	2.30^{b}	5.49^{a}
SEM	0.497	0.094	0.718	0.552	0.222	0.468	1.070	0.443	1.123	0.091	0.225
P value	0.281	<0.001	0.606	0.604	0.001	0.037	0.017	0.001	0.001	<0.001	<0.001
					Meal vs Pellet						
Contrast *P* value	0.188	0.004	0.472	0.477	0.766	0.014	0.008	0.001	0.001	<0.001	<0.001
					Chinese processing plants						
Plant A (M)	88.21	7.05	0.82	0.38	42.71^{bc}	9.53^{b}	22.33^{b}	4.74^{b}	11.07^{b}	2.14^{bc}	5.00^{bc}
Plant B (M)	88.54	7.09	0.41	0.02	43.31^{abc}	9.45^{b}	21.77^{bc}	7.05^{a}	16.27^{a}	2.85^{a}	6.60^{a}
Plant C (M)	88.52	7.42	0.74	0.35	43.25^{ab}	11.01^{a}	25.46^{a}	4.69^{b}	10.83^{b}	2.05^{c}	4.75^{c}
Plant D (M)	88.89	6.72	0.50	0.10	43.87^{a}	10.19^{ab}	23.24^{ab}	6.30^{a}	14.35^{a}	2.08^{c}	4.74^{c}
Plant E (M)	88.56	7.27	0.43	0.03	42.17^{c}	8.16^{c}	19.36^{c}	6.60^{a}	15.62^{a}	2.42^{b}	5.74^{b}
SEM	0.311	0.202	0.415	0.236	0.321	0.341	0.862	0.473	1.046	0.090	0.209
P value	0.615	0.112	0.554	0.599	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Overall						
CA Plants	89.96	7.89	0.79	0.46	41.87	7.15	17.11	8.07	19.34	2.45	5.86
CH Plants	88.55	7.12	0.47	0.16	43.04	9.71	22.51	5.83	13.52	2.29	5.33
SEM	0.285	0.143	0.397	0.211	0.305	0.365	0.851	0.48	1.125	0.104	0.245
P value	<0.001	<0.001	0.118	0.125	0.003	<0.001	<0.001	<0.001	<0.001	0.192	0.075

SEM, standard error of the mean. Means in the same row with different letters differ significantly (P < 0.05). CA: Canada; CH: China; DM, dry matter; CP, crude protein; SCP, soluble crude protein; NPN, non-protein nitrogen; NDICP, neutral detergent-insoluble crude protein; ADICP, acid detergent-insoluble crude protein; CHO, total carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Heme=Hemicellulose, calculated as NDF-ADF; Cell=Cellulose, calculated as ADF-ADL; NFC, non-fiber carbohydrate; NSC, non-structural carbohydrate.

Table 3.2. *(Cont'd.)* Chemical composition profile of co-products from different oil processing plants (canola meal and pellet): comparison among bio-oil processing plants and between Canada and China.

Items	Carbohydrate profile												
	CHO (%DM)	Sugar (%DM)	Sugar (%NFC)	NDF (%DM)	ADF (%DM)	ADF (%NDF)	ADL (%DM)	ADL (%NDF)	HEM (%DM)	Cell (%DM)	NFC (%DM)	NFC (%CHO)	NSC (%DM)
Canadian processing plants													
Plant 1 (M)	48.98	8.72	33.80	30.73^{bc}	20.03^{c}	65.10^{bc}	9.65^{bc}	31.62^{ab}	10.75^{a}	10.36^{b}	25.95	53.17^{ab}	14.20
Plant 2 (M)	50.14	7.97	31.06	33.26^{a}	21.90^{a}	66.31^{bc}	10.59^{a}	31.80^{a}	11.24^{a}	11.37^{a}	25.64	51.06^{b}	14.72
Plant 3 (P)	48.78	9.10	33.98	27.89^{d}	19.36^{c}	69.70^{ab}	7.92^{d}	28.28^{b}	8.45^{b}	11.48^{a}	26.99	55.22^{a}	14.87
Plant 4 (P)	49.81	9.58	36.70	29.92^{cd}	21.71^{ab}	72.70^{a}	9.96^{ab}	33.53^{a}	8.26^{b}	11.73^{a}	25.98	52.38^{ab}	14.42
Plant 5 (P)	50.38	8.06	31.50	32.66^{ab}	20.92^{b}	64.61^{c}	9.12^{c}	27.85^{b}	11.63^{a}	11.86^{a}	25.61	50.79^{b}	15.23
SEM	0.740	1.005	4.010	0.756	0.212	1.699	0.203	1.163	0.745	0.222	0.516	1.058	3.594
P value	0.046	0.583	0.721	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.355	0.018	0.531
Meal vs Pellet													
Contrast *P* value	0.788	0.442	0.573	<0.001	0.111	0.005	<0.001	0.011	0.002	<0.001	0.395	0.374	0.303
Chinese processing plants													
Plant A (M)	49.40	8.87^{ab}	35.00^{ab}	28.54^{b}	20.73	72.53^{a}	8.93^{ab}	31.18^{a}	7.83^{c}	11.83	25.63	51.61	15.35
Plant B (M)	49.25	8.88^{ab}	34.43^{b}	30.63^{ab}	21.46	70.34^{a}	9.76^{a}	31.86^{a}	9.11^{bc}	11.72	25.63	51.85	15.97
Plant C (M)	48.60	8.76^{b}	36.27^{ab}	29.06^{ab}	20.05	69.15^{ab}	8.17^{b}	28.19^{b}	9.01^{c}	11.87	24.22	49.87	15.34
Plant D (M)	48.91	10.21^{ab}	43.34^{ab}	31.65^{a}	20.51	64.85^{bc}	8.62^{b}	27.28^{b}	11.14^{ab}	11.87	23.57	48.17	14.99
Plant E (M)	50.02	10.91^{a}	43.65^{a}	31.62^{a}	20.25	64.07^{c}	8.77^{ab}	27.74^{b}	11.37^{a}	11.48	25.08	50.12	16.53
SEM	0.615	0.722	2.990	0.738	0.373	1.133	0.276	0.751	0.530	0.285	0.659	1.305	3.723
P value	0.143	0.019	0.009	0.012	0.062	<0.001	0.010	<0.001	<0.001	0.672	0.114	0.253	0.609
Overall													
CA Plants	49.48	8.44	33.09	31.74	20.86	65.83	10.07	31.80	10.88	10.80	25.81	52.24	14.34
CH Plants	49.41	9.56	38.74	30.62	20.56	67.91	8.81	29.07	9.80	11.75	24.76	50.20	14.69
SEM	0.562	0.688	2.540	0.633	0.302	1.226	0.228	0.758	0.542	0.190	0.441	0.819	3.212
P value	0.840	0.098	0.017	0.075	0.408	0.162	<0.001	0.005	0.103	<0.001	0.051	0.044	0.562

SEM, standard error of the mean. Means in the same row with different letters differ significantly (P < 0.05). CA: Canada; CH: China; DM, dry matter; CP, crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent-insoluble crude protein; ADICP: acid detergent-insoluble crude protein; CHO: total carbohydrate; NDF, neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; Heme: Hemicellulose, calculated as NDF-ADF; Cell: Cellulose, calculated as ADF-ADL; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrate.

Table 3.3. Chemical composition profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

	Basic chemical profile				Protein profile						
Items	DM (%)	Ash (%DM)	EE (%DM)	FA (%DM)	CP (%DM)	SCP (%DM)	SCP (%CP)	NDICP (%DM)	NDICP (%CP)	ADICP (%DM)	ADICP (%CP)
					Canadian processing plants						
Plant 1	93.67^{ab}	3.92^{a}	42.29	41.29	23.05	10.42^{bc}	45.18^{b}	2.67^{a}	11.60^{a}	1.18^{a}	5.14^{a}
Plant 2	94.83^{a}	3.69^{b}	40.66	39.66	22.09	9.43^{c}	42.84^{b}	2.60^{ab}	11.75^{a}	1.11^{a}	5.03^{a}
Plant 3	93.38^{bc}	3.97^{a}	44.79	43.79	22.81	10.28^{bc}	45.21^{b}	2.37^{b}	10.34^{a}	0.97^{b}	4.25^{b}
Plant 4	91.71^{d}	3.80^{ab}	43.42	42.42	22.14	11.70^{ab}	52.88^{a}	2.31^{b}	10.44^{a}	1.20^{a}	5.42^{a}
Plant 5	92.22^{cd}	3.80^{ab}	43.42	42.42	22.13	12.26^{a}	55.57^{a}	1.96^{c}	8.84^{b}	1.13^{a}	5.12^{a}
SEM	0.367	0.053	1.445	1.445	0.267	0.486	2.042	0.073	0.346	0.026	0.137
P value	<0.001	0.009	0.196	0.196	0.037	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Chinese processing plants						
Plant A	92.31^{ab}	3.77	43.09	42.09	22.48^{a}	12.49	55.53	2.05	9.15	1.06	4.69
Plant B	92.21^{bc}	3.72	46.06	45.06	21.70^{b}	12.24	56.39	2.02	9.34	1.18	5.41
Plant C	92.46^{ab}	3.87	43.07	42.07	22.40^{a}	12.07	54.04	2.00	8.94	1.11	4.97
Plant D	92.79^{a}	3.81	43.33	42.33	22.28^{a}	11.35	50.89	1.99	8.92	1.08	4.88
Plant E	92.71^{c}	3.83	44.37	43.37	22.18^{ab}	12.06	54.44	2.06	9.28	1.07	4.82
SEM	0.236	0.043	1.636	1.636	0.168	0.851	3.943	0.084	0.404	0.070	0.314
P value	<0.001	0.128	0.348	0.348	0.008	0.762	0.676	0.954	0.897	0.607	0.382
					Overall						
CA Plants	93.10	3.84	42.71	41.71	22.46	10.81	48.21	2.39	10.63	1.13	5.06
CH Plants	92.28	3.81	43.91	42.91	22.20	12.04	54.30	2.02	9.11	1.10	4.96
SEM	0.250	0.026	0.848	0.848	0.129	0.461	2.215	0.049	0.215	0.030	0.157
P value	0.008	0.387	0.191	0.191	0.100	0.003	0.002	<0.001	<0.001	0.338	0.537

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM, dry matter; CP, crude protein; SCP, soluble crude protein; NPN, non-protein nitrogen; NDICP, neutral detergent-insoluble crude protein; ADICP, acid detergent-insoluble crude protein; CHO, total carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Heme=Hemicellulose, calculated as NDF-ADF; Cell=Cellulose, calculated as ADF-ADL; NFC, non-fiber carbohydrate; NSC, non-structural carbohydrate.

Table 3.4. (*Cont'd.)* Chemical composition profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

Items	Carbohydrate profile												
	CHO (%DM)	Sugar (%DM)	Sugar (%NFC)	NDF (%DM)	ADF (%DM)	ADF (%NDF)	ADL (%DM)	ADL (%NDF)	HEM (%DM)	Cell (%DM)	NFC (%DM)	NFC (%CHO)	NSC (%DM)
Canadian processing plants													
Plant 1	30.74	4.95	30.51	17.04	12.18	71.55	5.32^{bc}	31.25^{ab}	4.85	6.86	16.37	53.06	9.24
Plant 2	33.61	4.85	26.54	17.44	12.45	71.30	5.53^{bc}	31.12^{ab}	5.05	6.97	18.63	54.91	10.80
Plant 3	28.48	5.74	39.20	16.27	12.03	73.69	4.94^{c}	29.81^{b}	4.29	7.15	14.44	51.02	9.04
Plant 4	30.65	5.29	35.99	17.52	14.41	76.68	6.44^{a}	36.84^{a}	4.11	6.96	15.44	50.05	10.07
Plant 5	30.71	5.74	34.42	15.94	12.31	77.24	5.89^{ab}	36.60^{a}	3.69	6.47	16.59	54.55	9.89
SEM	1.462	0.459	3.760	0.521	0.349	2.499	0.259	1.592	0.514	0.226	1.532	2.491	3.062
P value	0.122	0.490	0.165	0.147	0.048	0.285	0.001	0.009	0.336	0.282	0.227	0.364	0.824
Chinese processing plants													
Plant A	30.68	6.90	39.02	15.27	12.05	78.74	5.27	34.45	3.31	6.71	17.51	56.58	11.42
Plant B	28.50	5.69	38.90	15.59	12.17	78.86	5.85	37.53	3.36	6.26	14.89	51.99	10.03
Plant C	30.66	6.77	42.58	15.58	11.54	74.79	5.91	37.90	4.04	5.63	17.08	55.05	13.02
Plant D	30.57	5.56	34.73	15.91	11.44	72.59	5.64	35.67	4.47	5.80	16.65	54.22	11.22
Plant E	29.62	5.15	35.41	16.52	11.81	71.63	5.61	34.03	4.71	6.20	15.16	50.80	9.66
SEM	1.613	0.790	5.884	0.814	0.345	3.470	0.262	1.326	0.705	0.412	1.763	3.299	3.217
P value	0.585	0.368	0.721	0.477	0.468	0.062	0.246	0.111	0.064	0.328	0.387	0.300	0.140
Overall													
CA Plants	30.92	5.30	32.60	16.96	12.49	73.98	5.65	33.20	4.45	6.88	16.52	52.75	10.04
CH Plants	30.07	5.99	38.46	15.87	11.77	74.52	5.67	35.88	4.14	6.09	16.26	53.59	10.38
SEM	0.815	0.275	2.975	0.439	0.173	1.660	0.201	0.788	0.364	0.168	1.071	2.173	2.793
P value	0.361	0.076	0.032	0.004	0.003	0.762	0.920	0.017	0.387	<0.001	0.769	0.554	0.681

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM, dry matter; CP, crude protein; SCP, soluble crude protein; NPN, non-protein nitrogen; NDICP, neutral detergent-insoluble crude protein; ADICP, acid detergent-insoluble crude protein; CHO, total carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Heme=Hemicellulose, calculated as NDF-ADF; Cell=Cellulose, calculated as ADF-ADL; NFC, non-fiber carbohydrate; NSC, non-structural carbohydrate.

3.4.2. TDN and Digestible (DE), Metabolizable (ME) and Net Energy (NE) Values of Feedstocks and Co-products: Comparison among bio-oil processing plants and between two countries

The energetic profile of canola meals and pellets are represented in Table 3.5 and Table 3.6. Total digestible NDF (tdNDF), total digestible CP (tdCP), and total digestible nutrients (TDN_{1x}) were different among Canadian plants ($P<0.001$, $P=0.001$, and $P=0.001$, respectively). The contrast indicated that the meals pelleted (Plants 3, 4 and 5) resulted in higher tdNDF and TDN_{1x} ($P<0.001$, and $P=0.002$) than the mash. When pelleting, it is common practice to add back to the process fines collected during the screening step and that might have contributed to a lightly higher tdNDF in this study. Also, as a final step of pelleting, there is the spraying of oil to increase the durability of the pellet, which might have been the cause for a slightly higher TDN_{1x} on Plant 3. tdNDF and tdCP were also variable among the meals from Chinese plants ($P<0.001$ and $P=0.002$). When analyzing the overall meals from Canada and China, it was observed that tdNDF, tdNFC and tdCP were different ($P<0.001$, $P=0.006$, and $P<0.001$), of these, Canada had higher tdNFC, while China presented higher tdNDF and tdCP.

Metabolizable energy at three times maintenance (ME_{3x}), net energy for lactation (NE_{Lp3x}), maintenance (NE_{M3x}), and gain (NE_{g3x}) were all observed to be different among the meals from the Canadian plants ($P<0.001$, for all of them). Differences between mash and pelleted meals were also observed of these parameters ($P<0.05$) with the Plant 3 showing the higher results. While these differences were present on the Canadian samples, no differences were observed on the Chinese samples. Moreover, the overall comparison of canola meals from Canada and China showed that they are similar ($P>0.05$).

Damiran, Lardner, Jefferson, Karson, and McKinnon (2016) reported using canola meal with 42.6% of CP, 4.2% of fat, 71.5% of TDN, 2.0Mcal/g of NE_m, and 1.3Mcal/g of NE_g. While this study's Canadian canola meal averaged 41.9% of CP, 0.79% of EE, 65.6% of TDN, 1.8Mcal/g of NE_m, and 1.2Mcal/g of NE_g. Theodoridou and Yu (2013) analyzed canola meals from yellow and brown canola seeds and showed some differences in their energy profiles. Therefore, the higher TDN (71.5%) on Damiran et al. (2016) might be

explained by that canola meal being from a yellow seeded cultivar or as a consequence of the higher fat and protein content of that meal, since the TDN value is based on the values of digestible carbohydrates, protein and fat of a feedstuff (Tyrrell, 2005).

Table 3.5. Energy profile of co-products from different oil processing plants (canola meals and pellets): comparison among bio-oil processing plants and between Canada and China.

	Digestible nutrients profile (%DM)				
Items	tdNDF	tdNFC	tdCP	tdFA	TDN_{1x}
Canadian processing plants					
Plant 1 (M)	4.34^{c}	25.44	45.62^{a}	0.56	65.67^{b}
Plant 2 (M)	4.38^{c}	25.13	39.98^{b}	0.55	63.75^{b}
Plant 3 (P)	5.04^{ab}	26.45	40.84^{ab}	1.21	68.07^{a}
Plant 4 (P)	4.52^{bc}	25.46	40.74^{b}	0.83	65.59^{b}
Plant 5 (P)	5.63^{a}	25.09	40.94^{ab}	0.38	65.54^{b}
SEM	0.331	0.506	0.223	0.576	0.783
P value	<0.001	0.356	0.001	0.574	0.001
Meal vs. Pellet					
Contrast *P* value	<0.001	0.398	0.846	0.458	0.002
Chinese processing plants					
Plant A (M)	5.48^{ab}	25.12	41.85^{bc}	0.38	66.22
Plant B (M)	4.63^{b}	25.11	42.16^{abc}	0.02	65.00
Plant C (M)	6.29^{a}	23.74	42.43^{ab}	0.35	66.24
Plant D (M)	6.43^{a}	23.10	43.03^{a}	0.10	65.79
Plant E (M)	6.14^{a}	24.60	41.20^{c}	0.03	65.01
SEM	0.295	0.644	0.317	0.210	0.521
P value	<0.001	0.111	0.002	0.599	0.182
Chinese processing plants					
CA Plants	4.64	25.65	40.89	0.67	65.62
CH Plants	5.86	24.26	42.13	0.20	65.67
SEM	0.244	0.374	0.250	0.299	0.493
P value	<0.001	0.006	<0.001	0.055	0.926

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). M: meal; P: pellet; CA: Canada; CH: China; tdNDF: total digestible neutral detergent fiber; tdNFC: total digestible non-fiber carbohydrate; tdFA: total digestible fatty acids; TDN_{1x}: total digestible nutrients at one time maintenance level.

Table 3.6. *(Cont'd.)* Energy profile of co-products from different oil processing plants (canola meals and pellets): comparison among bio-oil processing plants and between Canada and China.

	Energy values (Mcal/Kg DM)			
Items	ME_{3x}	NE_{Lp3x}	NE_{m3x}	NE_{g3x}
	Canadian processing plants			
Plant 1 (M)	2.73[b]	1.75[ab]	1.81[ab]	1.18[ab]
Plant 2 (M)	2.65[c]	1.70[c]	1.73[c]	1.12[c]
Plant 3 (P)	2.81[a]	1.79[a]	1.87[a]	1.23[a]
Plant 4 (P)	2.72[b]	1.74[bc]	1.80[b]	1.17[bc]
Plant 5 (P)	2.72[b]	1.74[bc]	1.80[bc]	1.17[bc]
SEM	0.026	0.015	0.022	0.020
P value	<0.001	<0.001	<0.001	<0.001
	Meal vs Pellet			
Contrast *P* value	<0.001	0.003	0.001	0.002
	Chinese processing plants			
Plant A (M)	2.76	1.76	1.83	1.20
Plant B (M)	2.72	1.75	1.80	1.17
Plant C (M)	2.77	1.77	1.83	1.20
Plant D (M)	2.75	1.77	1.83	1.20
Plant E (M)	2.71	1.74	1.78	1.16
SEM	0.020	0.011	0.017	0.015
P value	0.086	0.066	0.071	0.106
	Overall			
CA Plants	2.73	1.75	1.80	1.17
CH Plants	2.74	1.76	1.82	1.19
SEM	0.019	0.010	0.016	0.014
P value	0.382	0.320	0.397	0.347

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; ME_{3x}: metabolizable energy for gain at three times the maintenance level; NEL_{p3x}: net energy for lactation at a productive level of intake three times the maintenance level; NEm_{3x}: net energy for maintenance; NEg_{3x}: net energy for gain.

The energy profile of canola seeds is displayed in Table 3.7 and Table 3.8. As expected, the seeds presented less variations. No differences were observed on the digestible nutrients profile from Canadian plants. Only the tdCP of canola seeds from the Chinese companies were different oin this study (P=0.006). The overall comparison of the energetic parameters of canola seeds from Canada and China only the tdNDF from Canadian plants were higher (P=0.023), while all the other parameters were similar. Similar values were observed for ME_{3x}, NE_{Lp3x}, NE_{m3x}, and NE_{g3x} on all samples collected in Canada and in China (P>0.05).

Table 3.7. Energy profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

Items	Digestible nutrients profile (%DM)				
	tdNDF	tdNFC	tdCP	tdFA	TDN_{1x}
Canadian processing plants					
Plant 1	3.29	16.05	22.58	41.29	127.82
Plant 2	3.47	18.26	21.66	39.66	125.55
Plant 3	3.44	14.15	22.43	43.79	131.49
Plant 4	2.89	15.12	21.66	42.42	128.11
Plant 5	2.75	16.25	21.69	42.42	129.06
SEM	0.246	1.502	0.268	1.445	1.743
P value	0.157	0.226	0.038	0.196	0.136
Chinese processing plants					
Plant A	2.75	17.16	22.06[a]	42.09	129.61
Plant B	2.52	14.60	21.23[b]	45.06	132.76
Plant C	2.45	16.74	21.95[a]	42.07	128.81
Plant D	2.84	16.31	21.85[a]	42.33	129.24
Plant E	3.11	14.86	21.75[ab]	43.37	130.30
SEM	0.279	1.728	0.180	1.636	2.098
P value	0.145	0.387	0.006	0.348	0.383
Overall					
CA Plants	3.15	16.18	22.01	41.71	129.75
CH Plants	2.77	15.93	21.76	42.91	128.07
SEM	0.146	1.049	0.135	0.848	1.275
P value	0.023	0.770	0.126	0.191	0.328

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). M: meal; P: pellet; CA: Canada; CH: China; tdNDF: total digestible neutral detergent fiber; tdNFC: total digestible non-fiber carbohydrate; tdFA: total digestible fatty acids; TDN_{1x}: total digestible nutrients at one time maintenance level

Table 3.8. *(Cont'd)* Energy profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

Items	Energy values (Mcal/Kg DM)			
	ME_{3x}	NEL_{p3x}	NE_{m3x}	NE_{g3x}
	Canadian processing plants			
Plant 1	4.64	3.08	3.31	2.41
Plant 2	4.55	3.02	3.25	2.36
Plant 3	4.76	3.18	3.41	2.48
Plant 4	4.64	3.09	3.32	2.41
Plant 5	4.67	3.11	3.34	2.43
SEM	0.060	0.048	0.046	0.036
P value	0.122	0.149	0.132	0.129
	Chinese processing plants			
Plant A	4.70	3.12	3.36	2.45
Plant B	4.79	3.21	3.44	2.50
Plant C	4.67	3.11	3.33	2.43
Plant D	4.68	3.12	3.34	2.44
Plant E	4.71	3.15	3.37	2.46
SEM	0.072	0.057	0.055	0.043
P value	0.461	0.426	0.390	0.454
	Overall			
CA Plants	4.65	3.09	3.32	2.42
CH Plants	4.71	3.14	3.36	2.45
SEM	0.034	0.028	0.026	0.020
P value	0.161	0.162	0.173	0.143

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; ME_{3x}: metabolizable energy for gain at three times the maintenance level; NEL_{p3x}: net energy for lactation at a productive level of intake three times the maintenance level; NEm_{3x}: net energy for maintenance; NEg_{3x}: net energy for gain.

3.4.3. Protein and Carbohydrate Subfractions and Degradable and Digestible Content of Each Fraction in Rumen Phase and Intestinal Phase Using Newly Updated CNCPS System 6.5 for Feedstocks and Co-Products

In Table 3.9 presents the protein fractions of canola meals and pellets based on the CNCPS 6.5 System. For the Canadian canola meals and pellets, it was observed that for the soluble fraction of protein (PA2) of the canola meals mash and pellets, the Plant 4 presented the highest amount (20.55%CP) while Plant 1 presented the lowest (16.63%CP). For the moderately degradable fraction (PB1), Plant 3 had the highest value (66.44%CP), and Plant 2 had the lowest (61.45%CP) among the companies. On the slowly degradable protein fraction (PB2), while Plant 2 resulted in the highest value for the fraction (15.44%CP), Plant 4 had the lowest (8.82%CP). Plant 3 presented the amount of unavailable protein (PC: 4.87%CP), whereas Plant 2 the highest (5.98%CP). The contrast analysis also showed differences between the mash and pelleted meals for soluble, slowly degradable, and unavailable fractions of protein (PA2: $P=0.008$; PB2: $P=0.003$; PC: $P<0.001$). Possibly, the conditioning step of the pelleting process, that uses high temperatures, influenced the protein structures of the meals, and consequently increased their availability for degradation. All fractions were different among the Chinese plants (PA2: $P<0.001$; PB1: $P=0.021$; PB2: $P<0.001$; PC: $P<0.001$). However, the comparison between the Canadian and Chinese protein fractions of the meals showed that only PA2 ($P<0.001$) and PB2 ($P<0.001$) were different, with China having higher soluble and lower slowly degradable fractions than Canada.

The ruminal degradable and undegradable, and intestinal digestible fractions profile of the Canadian and Chinese canola meals and pellets are shown in Table 3.15. In accordance with the results from Table 3.9, Table 3.15 shows that Plant 4 presented higher RDPA2 ($P=0.038$) and RUPA2 ($P=0.036$), and lower RDPB2 ($P=0.002$), RUPB2 ($P=0.002$), and DIGPB2 ($P=0.002$); and Plant 2 had lower RDPB1 ($P=0.003$), RUPB1 ($P=0.003$) and DIGPB1 ($P=0.003$). Because of the higher amounts of soluble true protein, Plant 4 presented lower amounts of intestinal digestible feed protein (18.69%DM, $P<0.001$). There were no differences between the meals and pellets on the amounts of RDPB1, RUPB1, and DIGPB1 fractions ($P>0.05$).

The Chinese meals presented variations in the ruminal degradability of PA2, PB2, peptides, and total ruminal degradable protein fractions ($P<0.001$, for all); on the ruminal undegradable PA2,

PB2, PC, and total rumen undegradable protein fractions (P<0.001, P<0.001, P<0.001, and P=0.006, respectively); and on the intestinal digestible PB2 and feed protein (DIGFP) fractions (P<0.001, and P=0.039).

While the rumen degradable fractions of PA2, PEP, and total RDP, and the rumen undegradable fraction of PA2 were higher in the Chinese meals (P<0.001), the rumen degradable PB2, rumen undegradable PB2, and intestinal digestible PB2 and FP fractions were higher for the Canadian meals. Higher availability of protein in the rumen (degradable fractions) guarantees enough amino acid supply for the rumen microbiota, however higher availability of protein for intestinal digestion and absorption (intestinal digestible fractions) means that a higher variability of amino acids will be available for the animal to use for muscle deposition and milk production.

The protein fractions of the canola seeds analyzed in this study are represented in Table 3.10. The Canadian seeds presented some variation on the contents of PB2, PC, and TP fractions (P<0.001, for all). The Canadian Plant 2 had the highest content of PB2 (6.72%), while Plant 5 presented the lowest (3.72%). Plant 4 showed higher content of PC (5.42%) and lower content of TP (94.58%). The opposite was observed on Plant 3 that showed the lowest PC (4.25%) and the highest TP (95.75%). All the seeds from the five different Chinese companies were similar for all protein fractions presented (P>0.05). Only the slowly degradable fraction (PB2) was different between Canada and China (P<0.001), where Canadian seeds presented higher amounts of this fraction.

The rumen and intestinal fractions are presented in Table 3.16, where we see a similar behavior. RDPB2, RUPB2, RUPC, and DIGPB2 are different among Canadian plants (all P<0.001). No difference is observed among the seeds from the various Chinese plants, and RDPB2, RUPB2, and DIGPB2 are higher in the seeds from Canada (P<0.001).

Li, Zhang, and Yu (2016) analyzing co-products from canola bio-energy processing found PA2: 26.8%CP, PB1: 63.6% CP, PB2: 7.0% CP, and PC: 2.6% CP. And predicted RDPA2: 7.7% DM, RDPB1: 13.9% DM, RDPB2: 0.7% DM. Total RDP: 22.3% DM, RUPA2: 2.6% DM, RUPB1: 10.5% DM, RUPB2: 2.0% DM, RUPC: 1.0% DM, and Total RUP: 16.1% DM. The values for PA2, RDPA2, RDPB1, and Total RDP are higher than the ones found for canola meals on this study. And their contents of PB2, PC, RDPB2, RUPB1, RUPB2, RUPC, and Total RUP are lower than ours. However, we had similar results for PB1 and RUPA2.

Table 3.9. CNCPS 6.5 protein fractions profile of co-products from different oil processing plants (canola meals and pellets): comparison among bio-oil processing plants and between Canada and China.

	%CP				%TP				%DM			
Items	PA2	PB1	PB2	PC	TP	PA2	PB1	PB2	PA2	PB1	PB2	PC
Canadian processing plants												
Plant 1 (M)	16.63^{b}	65.08^{ab}	12.17^{ab}	5.80^{a}	94.20^{b}	17.65^{b}	69.06^{ab}	12.88^{ab}	7.08^{b}	27.74^{a}	5.19^{abc}	2.47^{a}
Plant 2 (M)	17.19^{ab}	61.45^{b}	15.44^{a}	5.98^{a}	94.02^{b}	18.29^{ab}	65.43^{b}	16.40^{a}	7.05^{ab}	25.11^{b}	6.30^{a}	2.45^{ab}
Plant 3 (P)	19.00^{ab}	66.44^{a}	9.75^{b}	4.87^{b}	95.13^{a}	19.98^{ab}	69.90^{a}	10.25^{b}	7.92^{ab}	27.60^{a}	4.06^{bc}	2.02^{c}
Plant 4 (P)	20.55^{a}	64.62^{ab}	8.82^{b}	5.69^{a}	94.31^{b}	21.78^{a}	68.50^{ab}	9.31^{b}	8.57^{a}	26.95^{a}	3.67^{c}	2.37^{ab}
Plant 5 (P)	17.82^{ab}	63.40^{ab}	13.35^{ab}	5.49^{a}	94.51^{b}	18.86^{ab}	67.15^{ab}	14.11^{ab}	7.46^{ab}	26.47^{ab}	5.58^{ab}	2.30^{b}
SEM	1.070	1.155	1.216	0.225	0.225	1.100	1.327	1.290	0.468	0.517	0.487	0.090
P value	0.017	0.012	0.003	<0.001	<0.001	0.018	0.029	0.003	0.037	0.003	0.002	<0.001
Meal vs Pellet												
Contrast *P* value	0.008	0.053	0.003	<0.001	<0.001	0.010	0.124	0.003	0.014	0.110	0.002	<0.001
Chinese processing plants												
Plant A (M)	22.32^{b}	66.99^{a}	6.01^{b}	5.00^{bc}	95.00^{ab}	23.52^{bc}	70.49^{a}	6.34^{b}	9.53^{b}	28.52	2.58^{b}	2.14^{bc}
Plant B (M)	21.77^{bc}	62.16^{b}	9.66^{a}	6.60^{a}	93.40^{c}	23.31^{bc}	66.55^{ab}	10.34^{a}	9.45^{b}	26.86	4.18^{a}	2.85^{a}
Plant C (M)	25.46^{a}	63.71^{ab}	6.08^{b}	4.75^{c}	95.25^{a}	26.73^{a}	66.89^{ab}	6.39^{b}	11.01^{a}	27.56	2.63^{b}	2.05^{c}
Plant D (M)	23.24^{ab}	62.41^{b}	9.61^{a}	4.74^{c}	95.26^{a}	24.40^{ab}	65.52^{b}	10.08^{a}	10.19^{ab}	27.36	4.23^{a}	2.08^{c}
Plant E (M)	19.36^{c}	65.01^{ab}	9.88^{a}	5.74^{b}	94.26^{b}	20.54^{c}	68.97^{ab}	10.49^{a}	8.16^{c}	27.41	4.17^{a}	2.42^{b}
SEM	0.862	1.052	0.978	0.209	0.209	0.915	1.049	1.035	0.341	0.486	0.438	0.091
P value	<0.001	0.021	<0.001	<0.001	<0.001	<0.001	0.016	<0.001	<0.001	0.183	<0.001	<0.001
Overall												
CA Plants	17.11	63.48	13.48	5.86	94.14	18.18	67.44	14.32	7.15	26.22	5.62	2.45
CH Plants	22.51	64.01	8.18^{b}	5.33	94.67	23.77	67.61	8.65	9.71	27.54	3.53	2.29
SEM	0.851	0.939	1.023	0.245	0.245	0.875	0.988	1.089	0.365	0.443	0.435	0.104
P value	<0.001	0.636	<0.001	0.075	0.075	<0.001	0.887	<0.001	<0.001	0.082	<0.001	0.192

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; M: meal; P: pellet; TP: true protein; CP: crude protein; DM: dry matter; PA2: soluble true protein; PB1: moderately degradable protein; PB2: slowly degradable protein; PC: unavailable crude protein.

Table 3.10. CNCPS 6.5 protein fractions profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

	%CP				%TP				%DM			
Items	PA2	PB1	PB2	PC	TP	PA2	PB1	PB2	PA2	PB1	PB2	PC
						Canadian processing plants						
Plant 1	53.06	35.32	6.46^{a}	5.14^{a}	94.86^{b}	55.93	37.22	6.81^{a}	12.24	8.15	1.49^{a}	1.18^{a}
Plant 2	54.91	33.81	6.72^{a}	5.03^{a}	94.97^{b}	57.84	35.66	7.08^{a}	12.01	7.38	1.49^{a}	1.11^{a}
Plant 3	51.02	39.11	6.10^{ab}	4.25^{b}	95.75^{a}	53.30	40.90	6.37^{ab}	11.50	8.85	1.39^{ab}	0.97^{b}
Plant 4	50.05	39.49	5.02^{b}	5.42^{a}	94.58^{b}	52.91	41.74	5.31^{b}	11.09	8.74	1.11^{b}	1.20^{a}
Plant 5	54.55	37.09	3.72^{c}	5.12^{a}	94.89^{b}	57.50	39.16	3.92^{c}	11.94	8.12	0.83^{c}	1.13^{a}
SEM	2.410	2.870	0.308	0.137	0.137	2.619	2.997	0.325	0.741	0.779	0.068	0.026
P value	0.364	0.187	<0.001	<0.001	<0.001	0.341	0.194	<0.001	0.404	0.1994	<0.001	<0.001
						Chinese processing plants						
Plant A	56.58	34.23	4.34	4.69	95.31	59.33	35.92	4.56	12.72	7.71	0.97	1.06
Plant B	51.99	38.75	3.87	5.41	94.59	54.95	40.96	4.09	11.30	8.40	0.83	1.18
Plant C	55.05	36.02	3.97	4.97	95.03	57.89	37.94	4.17	12.35	8.04	0.89	1.11
Plant D	54.22	36.86	4.04	4.88	95.12	56.95	38.8	4.25	12.09	8.21	0.90	1.08
Plant E	50.80	39.93	4.46	4.82	95.18	53.36	41.96	4.68	11.27	8.86	0.99	1.07
SEM	3.298	3.199	0.477	0.314	0.314	3.341	3.447	0.493	0.784	0.700	0.109	0.066
P value	0.300	0.280	0.860	0.382	0.382	0.302	0.287	0.866	0.186	0.402	0.807	0.607
						Overall						
CA Plants	52.75	36.54	5.62	5.05	94.95	55.59	38.52	5.91	11.87	1.26	8.21	1.13
CH Plants	53.59	37.29	4.14	4.96	95.04	56.37	39.27	4.35	11.92	0.92	8.27	1.10
SEM	2.173	2.138	0.229	0.157	0.157	2.224	2.291	0.239	0.543	0.052	0.445	0.030
P value	0.554	0.588	<0.001	0.537	0.537	0.597	0.608	<0.001	0.874	<0.001	0.829	0.338

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM: dry matter; TP: true protein; DM: dry matter; PA2: soluble true protein; PB1: moderately degradable protein; PB2: slowly degradable protein; PC: unavailable crude protein.

The carbohydrate fractions of canola meals and pellets are given in Table 3.11. Canadian canola meals different among the five plants for digestible (CB3) ($P=0.002$) and indigestible fiber (CC) ($P<0.001$). Plant 4 showed the lowest amount of digestible fiber (CB3) and the highest of indigestible fiber (CC). Plant 5 displayed the highest content of CB3 and Plant 3 the lowest amount of CC. Only the CC fraction showed a difference between the mash and pelleted meals ($P<0.001$). The Chinese meals presented variability among companies on the CB2, CB3, and CC fractions ($P=0.012$, $P=0.013$, and $P=0.010$, respectively). The Chinese plant B showed higher quantities of CB2 and CC than the other companies. And Plant D had higher amount of CB3. Besides these differences, Canadian and Chinese meals were only different on the content of CA4, CB2, and CC ($P=0.040$, $P=0.010$, and $P<0.001$).

The predicted rumen degradable and undegradable and intestinal digestible carbohydrate fractions are revealed in Table 3.13. The rumen degradable CB3 (RDCB3), rumen undegradable CB3 (RUCB3), and intestinal digestible CB3 (DIBCB3) fractions were found to be the highest on Plant 5, and the lowest on Plant 2 ($P<0.001$, for all three). Total rumen undegradable carbohydrate (Total RUC) was the highest on Plant 2 (24.78% DM) and the lowest on Plant 3 (20.66% DM) ($P<0.001$). The intestinal digestible feed carbohydrate (DIGFC) was the highest on Plant 5 (13.11%) and the lowest on Plant 3 (11.44%DM). The contrast analysis showed that pelleting influenced the RUCC and Total RUC fractions of the canola meals on this study ($P<0.001$, for both). The rumen degradable and undegradable CA4, CB2 and CB3 fractions were variable among the Chinese plants ($P<0.05$). Plant E presented the highest values for RDCA4 and RUCA4 (8.4% DM, $P=0.018$; 2.52% DM, $P=0.018$, respectively). Plant B showed the highest amounts of RDCB2 (12.25% DM, $P=0.014$) and RUCB2 (3.68% DM, $P=0.014$). Plant D resulted in the highest contents of RDCB3, RUCB3, and DIGCB3 ($P=0.007$, for all). The rumen degradable, undegradable and intestinal digestible CB2, the RUCC, and Total RUC fractions of canola meals were higher in the Canadian companies ($P=0.009$, $P=0.008$, $P=0.008$, $P<0.001$, and $P=0.009$, respectively).

In Table 3.12 are expressed the carbohydrate fractions of canola seeds from Canadian and Chinese companies. Only the CB2 and CC fractions seemed to be different among companies ($P=0.002$ and $P<0.001$, respectively), where Plant 3 showed the lowest values for both (25.99% and 11.84% CHO). All the samples analyzed from the five Chinese samples were similar ($P>0.05$).

Only the amounts of water-soluble CHO (CA4) and digestible fiber (CB3) differed between countries (P=0.022 and P=0.006).

Table 3.14 shows the predicted amounts of rumen degradable and undegradable and intestinal digestible carbohydrate fractions of canola seeds. This table shows that while Plant 5 exhibited the highest values of rumen degradable, undegradable and intestinal digestible CB2, and Total RDC, the Plant 3 exhibited the lowest values for those variables (8.86% vs. 5.61% DM, P=0.003; 2.66% vs. 1.68% DM, P=0.003; 2.66% vs. 1.68% DM, P=0.003; and 17.21% vs. 14.52% DM, P=0.020, respectively). Apart from DIGFC (P=0.043), all other variables analyzed on the Chinese canola seeds were similar. And excluding the CB3 fractions (RDCB3, RUCB3, and DIGCB3, P=0.006 for these three), all other fractions are similar between the canola seeds analyzed from Canadian and Chinese companies.

Huang (2015) reported a study on different temperatures and conditioning time during the pelleting of canola meals and showed that neither the carbohydrate fractions nor the predicted rumen degradable and undegradable carbohydrate fractions were affected by the different treatments. This finding is in accordance with our results because only the indigestible fiber fractions (CC, RUCC, and Total RUC) expressed a difference between mash and pellets (P<0.001, for the three fractions).

Table 3.11. CNCPS 6.5 carbohydrate fractions profile of co-products from different oil processing plants (canola meals and pellets): comparison among bio-oil processing plants and between Canada and China.

Items	CHO	CA4	CB1	CB2	CB3	CC	CA4	CB2	CB3	CC
	%DM	%CHO					%DM			
Canadian processing plants										
Plant 1 (M)	48.98	17.95	2.04	33.17	23.92^{ab}	23.18^{bc}	8.72	16.27	11.76^{b}	11.32^{b}
Plant 2 (M)	50.14	15.69	1.99	32.94	24.50^{ab}	25.43^{a}	7.97	16.49	12.33^{b}	12.75^{a}
Plant 3 (P)	48.78	18.63	2.05	34.11	21.36^{b}	19.00^{d}	9.10	16.71	10.45^{b}	9.25^{c}
Plant 4 (P)	49.81	19.45	2.01	30.92	21.09^{b}	23.89^{ab}	9.58	15.43	10.55^{b}	11.89^{ab}
Plant 5 (P)	50.38	15.81	1.99	32.57	28.52^{a}	21.88^{c}	8.06	16.36	14.41^{a}	11.02^{b}
SEM	0.740	2.613	0.032	2.092	2.000	0.488	1.005	1.047	0.625	0.296
P value	0.046	0.609	0.073	0.785	0.002	<0.001	0.583	0.909	<0.001	<0.001
Meal vs Pellet										
Contrast *P* value	0.788	0.476	0.913	0.757	0.529	<0.001	0.442	0.812	0.531	<0.001
Chinese processing plants										
Plant A (M)	49.40	18.06	2.03	31.75^{ab}	22.19^{b}	21.44^{ab}	8.87^{ab}	15.67^{a}	10.82^{b}	10.59^{ab}
Plant B (M)	49.25	18.11	2.03	32.43^{a}	24.24^{ab}	23.42^{a}	8.88^{ab}	15.93^{a}	11.95^{ab}	11.52^{a}
Plant C (M)	48.60	18.07	2.06	29.75^{ab}	24.91^{ab}	19.62^{b}	8.76^{b}	14.46^{ab}	12.10^{ab}	9.54^{b}
Plant D (M)	48.91	20.91	2.04	25.21^{b}	30.32^{a}	20.69^{b}	10.21^{ab}	12.36^{b}	14.80^{a}	10.12^{b}
Plant E (M)	50.02	21.83	2.00	26.29^{ab}	27.68^{ab}	21.06^{ab}	10.91^{a}	13.17^{ab}	13.82^{ab}	10.52^{ab}
SEM	0.615	1.551	0.025	1.692	1.714	0.662	0.722	0.894	0.764	0.382
P value	0.143	0.019	0.153	0.012	0.013	0.010	0.019	0.014	0.007	0.008
Overall										
CA Plants	49.48	17.25	2.02	32.93	24.04	24.17	8.44	16.30	11.91	11.96
CH Plants	49.41	19.42	2.02	28.81	26.02	21.15	9.56	14.21	12.81	10.44
SEM	0.562	1.279	0.023	1.403	1.275	0.547	0.688	0.772	0.606	0.320
P value	0.840	0.040	0.906	0.010	0.200	<0.001	0.098	0.009	0.214	<0.001

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; M: meal; P: pellet; TP: true protein; CP: crude protein; DM: dry matter; CHO: carbohydrates; CA4: water soluble carbohydrate; CB2: soluble fiber; CB3: digestible fiber; CC: indigestible fiber.

Table 3.12. CNCPS 6.5 carbohydrate fractions profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

	%DM	%CHO				%DM			
Items	CHO	CA4	CB2	CB3	CC	CA4	CB2	CB3	CC
Canadian processing plants									
Plant 1	30.74	16.14	28.83^{bc}	30.04	12.76^{bc}	4.95	8.80^{ab}	9.21	3.91^{bc}
Plant 2	33.61	14.36	29.29^{bc}	28.58	13.26^{bc}	4.85	9.73^{ab}	9.42	4.35^{ab}
Plant 3	28.48	20.02	25.99^{c}	31.49	11.84^{c}	5.74	7.30^{b}	8.99	3.33^{c}
Plant 4	30.65	17.63	35.04^{ab}	29.55	15.46^{a}	5.29	10.75^{a}	8.98	4.75^{a}
Plant 5	30.71	18.71	37.66^{a}	25.40	14.14^{ab}	5.74	11.51^{a}	7.87	4.30^{ab}
SEM	1.462	1.578	2.150	2.218	0.622	0.459	0.692	0.496	0.224
P value	0.122	0.141	0.002	0.315	<0.001	0.500	0.003	0.248	0.003
Chinese processing plants									
Plant A	30.68	22.50	33.59	25.24	12.65	6.90	10.37	7.62	3.88
Plant B	28.50	19.68	36.88	27.96	14.05	5.70	10.45	7.88	3.99
Plant C	30.66	22.47	31.57	24.63	14.18	6.77	9.61	7.38	4.32
Plant D	30.57	18.35	32.54	26.2	13.53	5.56	9.96	7.95	4.15
Plant E	29.62	17.69	36.75	29.63	13.47	5.15	10.90	8.69	4.00
SEM	1.613	2.661	2.912	2.794	0.628	0.790	0.984	0.690	0.261
P value	0.585	0.457	0.331	0.258	0.245	0.368	0.600	0.254	0.358
Overall									
CA Plants	30.92	17.07	31.44	28.93	13.56	5.30	9.66	8.90	4.18
CH Plants	30.07	20.17	34.21	26.82	13.60	5.99	10.28	7.98	4.09
SEM	0.815	1.164	1.849	1.78	0.483	0.275	0.531	0.392	0.185
P value	0.361	0.022	0.077	0.107	0.920	0.076	0.250	0.006	0.595

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM: dry matter; TP: true protein; DM: dry matter; CHO: carbohydrates; CA4: water soluble carbohydrate; CB2: soluble fiber; CB3: digestible fiber; CC: indigestible fiber.

Table 3.13. CNCPS 6.5 carbohydrate ruminal profile of co-products from different oil processing plants (canola meal and pellet): comparison among bio-oil processing plants and between Canada and China.

	Rumen Degradable profile (%DM)				Rumen Undegradable profile (%DM)					Intestinal Digestible profile (%DM)			
Items	RDCA4	RDCB2	RDCB3	Total RDC	RUCA4	RUCB2	RUCB3	RUCC	Total RUC	DIGCA4	DIGCB2	DIGCB3	DIGFC
						Canadian processing plants							
Plant 1 (M)	6.71	12.51	5.88^{b}	25.90	2.01	3.75	5.88^{b}	11.32^{b}	23.25^{b}	2.01	3.75	5.88^{b}	11.90^{b}
Plant 2 (M)	6.13	12.69	6.16	25.87	1.84	3.81	6.16^{b}	12.75^{a}	24.78^{a}	1.84	3.81	6.16^{b}	12.07^{b}
Plant 3 (P)	7.00	12.86	5.22^{b}	25.97	2.10	3.86	5.22^{b}	9.25^{c}	20.66^{c}	2.10	3.86	5.22^{b}	11.44^{b}
Plant 4 (P)	7.37	11.87	5.28^{b}	25.31	2.21	3.56	5.28^{b}	11.89^{ab}	23.22^{b}	2.21	3.56	5.28^{b}	12.30^{b}
Plant 5 (P)	6.20	12.59	7.21^{a}	26.89	1.86	3.78	7.21^{a}	11.02^{b}	24.10^{ab}	1.86	3.78	7.21^{a}	13.11^{a}
SEM	0.773	0.904	0.312	0.492	0.232	0.270	0.312	0.260	0.449	0.232	0.270	0.312	0.314
P value	0.584	0.909	<0.001	0.109	0.579	0.908	<0.001	<0.001	<0.001	0.579	0.908	<0.001	<0.001
						Meal vs Pellet							
Contrast *P* value	0.443	0.814	0.536	0.600	0.440	0.809	0.536	<0.001	<0.001	0.440	0.809	0.536	0.827
						Chinese processing plants							
Plant A (M)	6.82^{ab}	12.05^{a}	5.41^{b}	25.13	2.05^{ab}	3.61^{a}	5.41^{b}	10.59^{ab}	22.00^{ab}	2.05^{ab}	3.61^{a}	5.41^{b}	11.33^{b}
Plant B (M)	6.83^{ab}	12.25^{a}	5.97^{ab}	25.68	2.05^{ab}	3.68^{a}	5.97^{ab}	11.52^{a}	23.47^{a}	2.05^{ab}	3.68^{a}	5.97^{ab}	11.89^{ab}
Plant C (M)	6.74^{b}	11.13^{ab}	6.05^{ab}	24.68	2.02^{b}	3.34^{ab}	6.05^{ab}	9.54^{b}	21.18^{b}	2.02^{b}	3.34^{ab}	6.05^{ab}	11.64^{ab}
Plant D (M)	7.86^{ab}	9.51^{b}	7.40^{a}	25.53	2.36^{ab}	2.85^{b}	7.40^{a}	10.12^{b}	22.97^{a}	2.36^{ab}	2.85^{b}	7.40^{a}	12.84^{a}
Plant E (M)	8.40^{a}	10.13^{ab}	6.91^{ab}	26.20	2.52^{a}	3.04^{ab}	6.91^{ab}	10.52^{ab}	23.23^{a}	2.52^{a}	3.04^{ab}	6.91^{ab}	12.70^{a}
SEM	0.555	0.688	0.381	0.387	0.166	0.206	0.381	0.382	0.435	0.166	0.206	0.381	0.317
P value	0.018	0.014	0.007	0.074	0.018	0.014	0.007	0.008	0.004	0.018	0.014	0.007	0.008
						Overall							
CA Plants	6.50	12.54	5.95	25.81	1.95	3.76	5.95	11.96	23.86	1.95	3.76	5.95	11.91
CH Plants	7.36	10.94	6.40	25.45	2.21	3.28	6.40	10.44	22.55	2.21	3.28	6.40	12.12
SEM	0.527	0.594	0.303	0.319	0.161	0.178	0.303	0.321	0.394	0.161	0.178	0.303	0.266
P value	0.097	0.009	0.215	0.344	0.101	0.008	0.215	<0.001	0.009	0.101	0.008	0.215	0.510

SEM, standard error of the mean. Means in the same column with different letters differ significantly (P < 0.05). CA: Canada; CH: China; M: meal; P: pellet; TP: true protein; DM: dry matter; RDCA4: rumen degradable water-soluble carbohydrates; RDCB2: RD soluble fiber; RDCB3: RD digestible fiber; Total RDC: total RD carbohydrates; RUCA4: rumen undegradable water soluble CHO; RUCB2: RU soluble fiber; RUCB3: RU digestible fiber; Total RUC: total RU CHO; RUCC: indigestible fiber; DIGCA4: digestible water-soluble CHO; DIGCB2: digestible soluble fiber; DIGCB3: digestible fiber; DIGFC: digestible feed CHO.

Table 3.14. CNCPS 6.5 carbohydrate ruminal profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

	Carbohydrate profile												
	Rumen Degradable profile				Rumen Undegradable profile					Intestinal Digestible profile			
Items	RDCA4 (%DM)	RDCB2 (%DM)	RDCB3 (%DM)	Total RDC (%DM)	RUCA4 (%DM)	RUCB2 (%DM)	RUCB3 (%DM)	RUCC (%DM)	TotalRUC (%DM)	DIGCA4 (%DM)	DIGCB2 (%DM)	DIGCB3 (%DM)	DIGFC (%DM)
Canadian processing plants													
Plant 1	3.80	6.77^{ab}	4.61	15.18^{ab}	1.14	2.03^{ab}	4.61	3.91^{bc}	11.71^{ab}	1.14	2.03^{ab}	4.61	7.77
Plant 2	3.73	7.48^{ab}	4.71	15.92^{ab}	1.12	2.24^{ab}	4.71	4.35^{ab}	12.41^{ab}	1.12	2.24^{ab}	4.71	8.08
Plant 3	4.42	5.61^{b}	4.50	14.52^{b}	1.33	1.68^{b}	4.50	3.53^{c}	10.82^{b}	1.33	1.68^{b}	4.50	7.51
Plant 4	4.07	8.27^{a}	4.49	16.83^{ab}	1.22	2.48^{a}	4.49	4.75^{a}	12.96^{a}	1.22	2.48^{a}	4.49	8.19
Plant 5	4.42	8.86^{a}	3.93	17.21^{a}	1.33	2.66^{a}	3.93	4.30^{ab}	12.20^{ab}	1.33	2.66^{a}	3.93	7.92
SEM	0.354	0.532	0.248	0.586	0.106	0.160	0.248	0.224	0.389	0.106	0.160	0.248	0.307
P value	0.487	0.003	0.244	0.020	0.481	0.003	0.244	0.003	0.010	0.481	0.003	0.244	0.523
Chinese processing plants													
Plant A	5.31	7.97	3.81	16.98	1.59	2.39	3.81	3.88	11.64	1.59	2.39	3.81	7.75^{ab}
Plant B	4.38	8.04	3.94	16.17	1.32	2.41	3.94	3.99	11.57	1.32	2.41	3.94	7.60^{ab}
Plant C	5.21	7.39	3.69	16.29	1.56	2.22	3.69	4.32	11.79	1.56	2.22	3.69	7.47^{b}
Plant D	4.28	7.66	3.98	15.92	1.28	2.30	3.98	4.15	11.70	1.28	2.30	3.98	7.56^{ab}
Plant E	3.96	8.38	4.35	16.69	1.19	2.52	4.35	4.00	12.04	1.19	2.52	4.35	8.05^{a}
SEM	0.608	0.757	0.345	1.023	0.182	0.226	0.345	0.261	0.611	0.182	0.226	0.345	0.452
P value	0.365	0.603	0.256	0.484	0.364	0.595	0.256	0.358	0.519	0.364	0.595	0.256	0.043
Overall													
CA Plants	4.07	7.43	4.45	15.94	1.22	2.23	4.45	4.18	12.15	1.22	2.23	4.45	7.90
CH Plants	4.61	7.91	3.99	16.52	1.38	2.37	3.99	4.09	11.82	1.38	2.37	3.99	7.75
SEM	0.211	0.409	0.196	0.524	0.063	0.122	0.196	0.185	0.379	0.063	0.122	0.196	0.265
P value	0.076	0.252	0.006	0.218	0.077	0.249	0.006	0.595	0.235	0.077	0.249	0.006	0.419

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM: dry matter; RDCA4: rumen degradable water-soluble carbohydrates; RDCB2: rumen degradable soluble fiber; RDCB3: rumen degradable digestible fiber; Total RDC: total rumen degradable carbohydrates; RUCA4: rumen undegradable water soluble carbohydrates; RUCB2: rumen undegradable soluble fiber; RUCB3: rumen undegradable digestible fiber; TotalRUC: total rumen undegradable carbohydrates; RUCC: indigestible fiber; DIGCA4: digestible water-soluble carbohydrates; DIGCB2: digestible soluble fiber; DIGCB3: digestible fiber; DIGFC: digestible feed carbohydrate.

Table 3.15. CNCPS 6.5 protein ruminal profile of co-products from different oil processing plants (canola meal and pellet): comparison among bio-oil processing plants and between Canada and China.

Items	Rumen Degradable profile					Rumen Undegradable profile					Intestinal Digestible profile		
	(%DM)	(%DM)	(%DM)	(%DM)	(%DM)	(%NDF)	(%DM)	(%DM)	(%DM)	(%DM)	(%DM)	(%DM)	(%DM)
	RDPA2	RDPB1	RDPB2	RDPEP	Total RDP	RUPA2	RUPB1	RUPB2	RUPC	Total RUP	DIGPB1	DIGPB2	DIGFP
						Canadian processing plants							
Plant 1 (M)	5.06^{b}	11.10^{a}	1.73^{abc}	17.92	17.92	2.03^{b}	16.65^{a}	3.46^{abc}	2.47^{a}	24.68^{a}	16.65^{a}	3.46^{abc}	20.18^{a}
Plant 2 (M)	5.04^{ab}	10.04^{b}	2.10^{a}	17.27	17.27	2.01^{ab}	15.07^{b}	4.20^{a}	2.45^{ab}	23.72^{bc}	15.07^{b}	4.20^{a}	19.27^{bc}
Plant 3 (P)	5.66^{ab}	11.04^{a}	1.35^{bc}	18.14	18.14	2.26^{ab}	16.56^{a}	2.70^{bc}	2.02^{c}	23.55^{c}	16.56^{a}	2.70^{bc}	19.27^{bc}
Plant 4 (P)	6.12^{a}	10.78^{a}	1.22^{c}	18.17	18.17	2.45^{a}	16.17^{a}	2.45^{c}	2.37^{ab}	23.51^{c}	16.17^{a}	2.45^{c}	18.69^{c}
Plant 5 (P)	5.33^{ab}	10.59^{ab}	1.86^{ab}	17.86	17.86	2.13^{ab}	15.88^{ab}	3.72^{ab}	2.30^{b}	24.02^{b}	15.88^{ab}	3.72^{ab}	19.61^{ab}
SEM	0.334	0.207	0.162	0.240	0.240	0.134	0.311	0.325	0.091	0.165	0.311	0.325	0.199
P value	0.038	0.003	0.002	0.074	0.074	0.036	0.003	0.002	<0.001	<0.001	0.003	0.002	<0.001
						Meal vs Pellet							
Contrast P value	0.014	0.109	0.002	0.034	0.034	0.014	0.112	0.003	<0.001	<0.001	0.112	0.003	0.001
						Chinese processing plants							
Plant A (M)	6.81^{b}	11.44	0.86^{b}	19.08^{bc}	19.08^{bc}	2.72^{b}	17.16	1.72^{b}	2.14^{bc}	23.68^{ab}	17.16	1.72^{b}	18.81^{ab}
Plant B (M)	6.75^{b}	10.75	1.39^{a}	18.88^{c}	18.88^{c}	2.70^{b}	16.12	2.79^{a}	2.85^{a}	24.47^{a}	16.12	2.79^{a}	18.91^{ab}
Plant C (M)	7.86^{a}	11.02	0.88^{b}	19.76^{a}	19.76^{a}	3.14^{a}	16.53	1.75^{b}	2.05^{c}	23.49^{b}	16.53	1.75^{b}	18.29^{b}
Plant D (M)	7.28^{ab}	10.95	1.41^{a}	19.63^{ab}	19.63^{ab}	2.91^{ab}	16.42	2.82^{a}	2.08^{c}	24.23^{a}	16.42	2.82^{a}	19.24^{a}
Plant E (M)	5.83^{c}	10.95	1.39^{a}	18.19^{d}	18.19^{d}	2.33^{c}	16.45	2.78^{a}	2.42^{b}	23.98^{ab}	16.45	2.78^{a}	19.23^{a}
SEM	0.244	0.194	0.146	0.125	0.125	0.098	0.292	0.292	0.091	0.269	0.292	0.292	0.329
P value	<0.001	0.186	<0.001	<0.001	<0.001	<0.001	0.185	<0.001	<0.001	0.006	0.185	<0.001	0.039
						Overall							
CA Plants	5.11	10.64	1.87	17.64	17.64	2.04	15.96	3.74	2.45	24.23	15.96	3.74	19.73
CH Plants	6.94	11.02	1.18	19.12	19.12	2.77	16.53	2.35	2.29	23.91	16.53	2.35	18.87
SEM	0.261	0.177	0.145	0.210	0.210	0.104	0.266	0.290	0.104	0.233	0.266	0.290	0.252
P value	<0.001	0.082	<0.001	<0.001	<0.001	<0.001	0.083	<0.001	0.192	0.139	0.083	<0.001	0.002

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; M: meal; P: pellet; TP: true protein; DM: dry matter; RDPA2: rumen degradable soluble true protein; RDPB1: RD moderately degradable protein; RDPB2: RD slowly degradable protein; RDPEP: RD peptides; TotalRDP: total RD protein; RUPA2: rumen undegradable soluble true protein; RUPB1: RU moderately degradable protein; RUPB2: RU

slowly degradable protein; RUPC: RU unavailable crude protein; TotalRUP: total RU unavailable protein; DIGPA2: digestible soluble protein; DIGPB1: moderately degradable protein; DIGPB2: digestible slowly degradable protein; DIGFP: digestible feed protein.

Table 3.16. CNCPS 6.5 protein ruminal profile of canola seeds from different oil processing plants: comparison among bio-oil processing plants and between Canada and China.

Items	Rumen Degradable profile					Rumen Undegradable profile					Intestinal Digestible profile		
	RDPA2 (%DM)	RDPB1 (%DM)	RDPB2 (%NFC)	RDPEP (%DM)	TotalRDP (%DM)	RUPA2 (%NDF)	RUPB1 (%DM)	RUPB2 (%NDF)	RUPC (%DM)	TotalRUP (%DM)	DIGPB1 (%DM)	DIGPB2 (%CHO)	DIGFP (%DM)
Canadian processing plants													
Plant 1	8.74	3.26	0.49^{a}	12.50	12.50	3.50	4.89	0.99^{a}	1.18^{a}	10.56	4.89	0.99^{a}	5.89
Plant 2	8.58	2.95	0.50^{a}	12.03	12.03	3.44	4.43	0.99^{a}	1.11^{a}	9.97	4.43	0.99^{a}	5.38
Plant 3	8.22	3.54	0.47^{ab}	12.21	12.21	3.29	5.31	0.93^{a}	0.97^{b}	10.50	5.31	0.93^{a}	6.20
Plant 4	7.92	3.50	0.37^{b}	11.79	11.79	3.17	5.24	0.74^{b}	1.20^{a}	10.35	5.24	0.74^{b}	5.99
Plant 5	8.53	3.25	0.27^{c}	12.05	12.05	3.42	4.88	0.55^{c}	1.13^{a}	9.98	4.88	0.55^{c}	5.38
SEM	8.739	0.293	0.023	0.267	0.267	0.215	0.429	0.046	0.027	0.187	0.429	0.046	0.330
P value	0.408	0.197	<0.001	0.137	0.137	0.405	0.199	<0.001	<0.001	0.074	0.199	<0.001	0.193
Chinese processing plants													
Plant A	9.08	3.08	0.32	12.50	12.50	3.63	4.62	0.65	1.06	9.99	4.62	0.65	5.31
Plant B	8.07	3.36	0.28	11.71	11.71	3.23	5.04	0.56	1.18	10.00	5.04	0.56	5.60
Plant C	8.82	3.22	0.30	12.34	12.34	3.53	4.83	0.59	1.11	10.06	4.83	0.59	5.42
Plant D	8.64	3.28	0.30	12.22	12.22	3.46	4.93	0.60	1.08	10.06	4.93	0.60	5.52
Plant E	8.05	3.54	0.33	11.92	11.92	3.22	5.31	0.66	1.07	10.26	5.31	0.66	5.97
SEM	0.560	0.276	0.036	0.306	0.306	0.224	0.414	0.073	0.066	0.221	0.414	0.073	0.396
P value	0.187	0.404	0.830	0.068	0.068	0.190	0.404	0.809	0.607	0.666	0.404	0.809	0.406
Overall													
CA Plants	8.48	3.28	0.42	12.18	12.18	3.39	4.92	0.84	1.13	10.31	4.92	0.84	5.77
CH Plants	8.51	3.31	0.31	12.13	12.13	3.41	4.97	0.61	1.10	10.09	4.97	0.61	5.58
SEM	0.389	0.178	0.017	0.196	0.196	0.155	0.267	0.035	0.030	0.128	0.267	0.035	0.254
P value	0.880	0.832	<0.001	0.740	0.740	0.883	0.827	<0.001	0.338	0.072	0.827	<0.001	0.332

SEM, standard error of the mean. Means in the same column with different letters differ significantly ($P < 0.05$). CA: Canada; CH: China; DM: dry matter; RDPA2: rumen degradable soluble true protein; RDPB1: rumen degradable moderately degradable protein; RDPB2: rumen degradable slowly degradable protein; RDPEP: rumen degradable peptides; TotalRDP: total rumen degradable protein; RUPA2: rumen undegradable soluble true protein; RUPB1: rumen undegradable moderately degradable protein; RUPB2: rumen undegradable slowly degradable protein; RUPC: rumen undegradable unavailable crude protein; TotalRUP: total rumen undegradable unavailable protein; DIGPA2: digestible soluble protein; DIGPB1: moderately degradable protein; DIGPB2: digestible slowly degradable protein; DIGFP: digestible feed protein.

3.5. Chapter summary

The chemical profile of canola meals from Canada and China presented some differences on DM, ash, CP, SCP, and NDICP ($P<0.001$, $P<0.001$, $P=0.003$, $P<0.001$, and $P<0.001$, respectively). Whereas the chemical profile of canola seeds from Canada and China presented some differences on DM, SCP, NDICP, NDF, AF, ADL, and Cellulose ($P=0.008$, $P=0.003$, $P<0.001$, $P=0.004$, $P=0.003$, $P=0.017$, $P<0.001$, respectively). Because variations can be caused by crop environment, cultivar, and processing, these differences do not seem relevant.

The pelleting of canola meals by the Canadian companies seemed to have influenced tdNDF and TDN_{1x} ($P<0.001$ and $P=0.006$). On the other hand, the meals from China were not pelleted and differences were observed on tdNDF and tdCP ($P<0.001$, and $P=0.002$). On the overall comparison of the mash meals, China presented higher tdNDF, and tdCP ($P<0.001$, for both), and lower tdNFC ($P=0.006$) than Canada.

The energy profile of canola seeds was very similar among companies on Canada and China except for tdCP on the Chinese samples that showed some variations among plants ($P=0.006$). Between countries, only tdNDF was higher in Canada ($P=0.023$). No differences were observed on the energy values (ME_{3x}, NE_{Lp3x}, NE_{m3x}, and NE_{g3x}) of canola seeds from China or Canada ($P>0.05$).

The protein fractions of the canola meals from Canada and China were similar, except for PA2 ($P<0.001$) and PB2 ($P<0.001$), where PA2 was higher in China and PB2 in Canada. The content of PB2 was also higher for the Canadian seeds ($P<0.001$). RDPA2, RUPA2, RDPEP, and Total RDP were higher on the Chinese meals (all $P<0.001$), whereas RDPB2, RUPB2, DIGPB2, and DIGPF were higher on the Canadian meals (in order, $P<0.001$, $P<0.001$, $P<0.001$, and $P=0.002$). While the Chinese seeds presented higher amounts of RDPB2, RUPB2, and DIGPB2 ($P<0.001$, for all).

The Chinese meals and seeds showed higher content of water-soluble carbohydrates (CA4) ($P=0.040$ and $P=0.022$, respectively). Canadian meals presented higher soluble (CB2) and indigestible (CC) fiber contents ($P=0.010$ and $P<0.001$), and consequently higher RDCB2, RUCB2, RUCC, and DIGCB2 than the ones from China ($P=0.009$, $P=0.008$, and $P=0.008$). The

meals from Canada were also higher in RUCC and Total RUC ($P<0.001$ and $P=0.009$, respectively). While the rumen degradable, undegradable and intestinal digestible fractions of CB3 were higher in Canada ($P=0.006$, for all), all the other variables were similar between the two countries.

From this study we can conclude that the canola seeds used by the companies from both countries are not different, and that the canola meals can present some variations depending on the processing it went through in the plant.

4. RUMINAL DEGRADATION AND INTESTINAL DIGESTION OF CANOLA SEEDS AND CANOLA MEALS AND PELLETS AND CHARACTERIZATION OF NUTRIENT SUPPLY (NRC AND DVE/OEB SYSTEMS) TO DAIRY COWS: COMPARISON BETWEEN CANADA AND CHINA

4.1. Abstract

The ruminal degradation and intestinal digestion of canola seeds and meals are important aspects to be considered on dairy production. Samples of canola seeds and meals from five crushing plants in Canada and five in China were used in this study. Four fistulated Holstein cows on second lactation were used for the *in situ* incubation study at the University of Saskatchewan. For the *in situ* study replicated samples were incubated at 0, 2, 4, 8, 12, 24, and 48h and the ruminal degradations kinetics of CP and DM were determined. Residues from the 12h incubations were later used for the three-step *in vitro* incubation to determine the intestinal digestibility of CP and DM. And the NRC and DVE/OEB models were used to predict the truly absorbable nutrient supply and feed milk values (FMV) for dairy cows. Undegradable fractions (U) (P=0.025) were higher in Canadian meals, and D was higher (P=0.016) in Chinese meals. Both countries presented similar hourly degradations of CP and DM for seeds and meals, except for CP of canola meals at 24h (P=0.042) and 48h (P=0.040) that CH was higher. The *in vitro* intestinal digestibility of DM and CP of the canola meals from CH resulted in higher TDDM (P=0.018) and dIDP (P=0.0.16). Canola meals from CA had lower amounts of MREE and DVME (P=0.011 and P=0.011) and had higher contents of ECP and AECP than CH (P=0.001 and P=0.001). The FMV evaluated based on the NRC, DVE/OEB systems, and energy showed no differences between countries for either canola meals or seeds.

4.2. Introduction

While canola seeds are primarily produced in Canada for oil for human consumption, its rich in protein co-product is extensively used for animal feed. The ideal amino acid profile and the high amount of bypass protein in canola meal turned this co-product an important ingredient for dairy rations. (Canola Council of Canada (CCC), n.d.). Canola meal can improve milk production and reduce methane production because of how efficiently cows can use the protein from canola (Beauchemin, McGinn, Benchaar, and Holtshausen, 2009).

Because of the microflora in the rumen, nutrient utilization in ruminants is different from other mammals (Virtanen, 1966) and the use of protein in the rumen needs to be considered correctly for overestimations of protein absorption can occur and the expected production is not equivalent to the reality. Therefore, in dairy production, more important than the quantity of protein included in the ration is the quality of this protein. How much of that protein is degraded in the rumen and how much can be truly absorbed in the small intestine are important aspects of a protein source fed to dairy cows.

In situ and *in vitro* studies have been used to determine the degradability and digestibility of nutrients in ruminants (Orskov and McDonald, 1979; Hvelplund, 1985; Damiran et al., 2013; Calsamiglia and Stern, 1995; Gargallo et al., 2006; Wang et al., 2015). Also, nutrient supply systems have been developed based on the chemical composition of an ingredient or feed, and on the dynamics of the rumen to further provide information regarding the utilization of that ingredient for animal production. Nutrient supply systems, for instance, use aspects of the chemical composition of an ingredient to predict the quantities of protein that is degraded or undegraded in the rumen, the amount that can be used for microbial protein synthesis, etc. These systems can also be used to predict the amount of milk produced based on that ingredient, called feed milk value (FMV). According to Theodoridou and Yu (2013) the amount of microbial protein synthesis and digestible protein in the small intestine are crucial for the efficiency in milk production.

The objective of this study was to determine the ruminal degradability, intestinal digestibility, the feed milk value, and the true nutrient supply based on the NRC and DVE/OEB Systems of canola seeds and canola meals and pellets comparing Canada and China.

4.3. Materials and Methods

4.3.1. Samples

The samples of feedstocks and co-products from bio-oil processing were collected from Canada and China by the Canola Council of Canada in 2016. The samples were provided by each company's quality control laboratory and are to be considered representative of the reality of those crushers.

Samples were collected from five crusher companies operating in four provinces in China. These companies only crushed seeds imported from Canada. Samples of seeds and meals were collected from different batches from each crusher, stored and transported to the University of Saskatchewan in Canada for further analyses.

Samples of seeds and meals were also collected from five crushers in Canada. However, three of the five Canadian crushers samples of meals were pelleted and two were mash, like China's meals that were all mash. Samples were collected from different batches from each crusher, stored and transported to the University of Saskatchewan for future analyses.

All samples of seeds were ground using a blade coffee grinder, model BCG111OB manufactured by KitchenAid®, USA. The samples of meals that were pelleted were ground using a 1mm screen on the grinding mill, Ultra Centrifugal Mill ZM200 manufactured by Retsch®, Germany.

4.3.2. Nylon bag in situ incubation procedure

The University of Saskatchewan Animal Research Ethics Board approved the use of the animals for this study. Four rumen fistulated Holstein cows on 2nd lactation in a tie-stall housing system were used for the *in situ* incubation study. The animals were housed in the Rainer Dairy

Research Facility at the University of Saskatchewan, Canada. This procedure was based on Orskov, Hovell and Mould (1980).

Samples of 7g were weighed into number coded nylon bags (10x20 cm; 41 µm). The bags were tied about 2 cm below the top with a string. Samples were incubated for 0, 2, 4, 8, 12, 24, and 48 hours. Samples were incubated in duplicate for the 0, 2, 4, and 8h time-points, and in triplicate for 12, 24, and 48h time-points to ensure enough residue would be available for the further procedures. The samples were incubated in a 'gradual addition/all out' schedule in four batches, never exceeding 30 bags per rumen.

On each time-point randomly selected nylon bags were inserted into a laundry mesh bag containing a heavy bottle (used as an anchor) and placed in the ventral sac of the rumen. Once the laundry bag was inside the rumen, it was only removed after the 48 hours of incubation. Only the zipper was open to insert the new bags at each time-point. This procedure reduced the exposure of the rumen to the room air.

After the incubation, the samples were immediately rinsed with cold water, until the water ran clear and then dried at 55°C for 48 hours in a forced-air drying oven. Rinsing halts fermentation and remove ruminal fluids and particles. Upon removal from the oven, samples were left exposed to room conditions for 24 hours to cool. Finally, the bags were weighed, and the amount of residue calculated. The bags were then pooled by treatment and incubation time, emptied and ground through a 1 mm screen using the laboratory Ultra Centrifugal Mill ZM200 (Retsch®, Germany) for dry matter (AOAC 930.15) and protein analysis by combustion method (AOAC 990.03). For the combustion nitrogen determination, the LECO P-528 Model machine was used (LECO Corporation, USA). The data was processed by SAS® 9.4 (SAS Institute, USA) using procedure NLIN with the iterative least square regression Gauss–Newton method to calculate and generate Kd (degradation rate) and T0 (lag time) values.

4.3.3. Three-step in vitro procedure for intestinal digestion study

The methodology for this procedure was adapted from Calsamiglia and Stern (1995) and Gargallo, Calsamiglia and Ferret (2006). The residues from the 12h rumen incubation were used.

0.3g of the 12h residue was weighed into a 50ml centrifuge tube. Pepsin solution (P-7000, Sigma) (0.1*N* HCl, pH 1.9), 1*N* NaOH solution, pancreatin (P-7545, Sigma) in a phosphate buffer solution, and trichloroacetic solution (TCA) were the reagent solutions used in this procedure. At the end of the procedure the supernatant was collected, and nitrogen was analyzed by the Kjeldahl method (AOAC Official Method 984.13, 2019).

4.3.4. NRC 2001 Model

The Nutrient Requirements of Dairy Cattle (NRC, 2001) was followed to calculate the nutrient supply to dairy cows in this study.

4.3.5. DVE/OEB System

The nutrient supply based on the DVE/OEB system followed the methodology published by Tamminga et al. 1994.

4.3.6. Feed milk value (FMV)

Feed milk values were estimated based on the NRC model, the DVE/OEB System and the energy available for milk production (Rodriguez, 2018).

4.3.7. Statistical Analysis

This study utilized a Complete Randomized Block Design (RCBD), with country, company and period as fixed effects and batch as random effect. The procedure MIXED was used on SAS® 9.4 (SAS Institute, USA).

$$y = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

Where, μ= overall mean; τ_i= fixed effect; β = random effect; ϵ_{ij}= error.

β_j ~ NIID (Normally, Identically, and Independently distributed)

ϵ_{ij} ~ NIID (Normally, Identically, and Independently distributed)

Significance was declared when $P<0.05$. The Tukey method was used for the multiple comparison test.

4.4 Results and Discussion

4.4.1. Rumen degradation kinetics of DM and CP of feedstocks and co-products from bio-oil processing: comparison among bio-oil processing plants and between two countries

The parameters of the *in situ* crude protein digestibility is in Table 4.1 and Table 4.3. The *in situ* CP parameter of canola meals were similar for Canadian meals and pellets (Table 4.1). They were also similar for the Chinese companies' meals, except the EDCP (effective degraded CP) that presented some variations (P=0.032). Despite the variation on China's EDCP, the average was not different from Canada's, as well as all other variables were also similar between countries (P>0.05).

Ebrahimi, Nikkhah, Sadeghi, and Raisali (2009) reported the effective degradation of DM and CP for different outflow rates (0.02, 0.05, and 0.08/h). Although we assumed the rate of passage for canola seeds and meals to be 0.06/h we still can compare our results. Their EDDM at 0.05 and 0.08/h rates were 75.5 and 69.5%, while ours at 0.06/h was 69.2%. They also reported EDCP at 0.05 and 0.8/h as 82.5 and 76.7%, whereas in our study we found EDCP for canola seeds at 0.06/h rate of passage as 79.1%. Therefore, Ebrahimi et al. (2009) found a higher degradability of EDDM and similar degradability of EDCP when compared to this study. According to Deacon, De Boer and Kennelly (1988) the oil content of the seed can influence the disappearance of DM in the rumen. Consequently, the lower degradability of DM on this study can be related to the higher oil content of 43.31% as Ebrahimi et al. (2009) reported their seeds had 41.9%.

The degradable fraction of CP (D) of canola seeds seemed to be variant among Canadian companies (P=0.045) (Table 4.3). None of the other parameters studied have shown any differences between the two countries (P>0.05).

The aspects of ruminal digestibility of DM of canola meals and pellets and seeds are shown in Table 4.2 and Table 4.4. No differences were observed among the Canadian companies, neither were they observed among the Chinese companies (P>0.05). However, rumen degradable fraction (D) and rumen undegradable fraction (U) were different between countries (P=0.016, and P=0.025, respectively). U was higher in Canadian meals and D was higher in Chinese meals (Table 4.2).

The in situ DM parameter of canola seeds were not different between countries ($P>0.05$), but the rate of digestion (Kd) was different among Canadian companies ($P=0.017$), and the rumen bypass or rumen undegraded dry matter and the effective degraded dry matter were different among Chinese companies ($P=0.005$, for both).

Table 4.1. In situ CP parameters of canola meals and pellets: comparisons between companies, and countries.

Items	Kd (%/h)	Fr (%)	T0 (h)	Residue at 0h (%)	S (%)	D (%)	U (%)	%BCP (%RUP)	BCP (g/kg DM)	%RUP	%EDCP	EDCP (g/kg DM)
Canadian processing plants												
Plant 1 (M)	8.26	6.76	0.22	86.00	14.01	80.18	5.81	39.57	188	169.78	60.43	259
Plant 2 (M)	5.51	6.28	0.00	81.38	18.62	76.27	5.09	44.88	205	184.80	55.12	227
Plant 3 (P)	7.32	7.11	0.00	88.80	11.20	82.49	6.29	43.48	201	181.19	56.52	235
Plant 4 (P)	6.14	7.29	0.00	87.46	12.54	81.14	6.33	46.81	215	194.46	53.20	221
Plant 5 (P)	5.75	5.29	0.00	91.94	8.06	87.07	4.84	49.31	230	207.22	50.69	213
SEM	0.947	2.536	0.215	2.054	2.054	3.997	3.577	3.050	11.5	10.324	3.050	15.6
P value	0.328	0.964	0.826	0.226	0.226	0.562	0.968	0.332	0.289	0.290	0.332	0.343
Meals vs. Pellets												
SEM	4.592	12.294	1.042	9.957	9.957	19.378	10.471	14.787	55.5	50.048	14.787	75.8
P value	0.592	0.986	0.599	0.075	0.075	0.240	0.867	0.223	0.178	0.179	0.223	0.256
Chinese processing plants												
Plant A (M)	8.90	4.53	0.01	91.52	8.48	88.24	4.26	39.93	193	173.99	60.07	261[ab]
Plant B (M)	7.56	3.76	-0.01	93.69	6.31	89.33	3.38	42.86	203	183.70	57.14	244[b]
Plant C (M)	9.36	5.29	0.00	85.68	14.32	81.17	4.52	36.24	175	157.90	63.76	277[a]
Plant D (M)	9.64	5.18	0.14	86.51	13.49	82.01	4.51	36.33	179	161.84	63.67	283[a]
Plant E (M)	7.73	2.79	0.07	92.42	7.59	89.84	2.58	41.84	197	178.06	58.16	247[b]
SEM	1.303	3.621	0.066	1.823	1.823	2.613	3.207	1.545	7.5	6.772	1.545	3.7
P value	0.573	0.976	0.596	0.079	0.078	0.154	0.984	0.196	0.138	0.138	0.196	0.032
Overall												
CA Plants	7.34	6.24	0.16	84.46	15.54	79.14	5.27	41.34	194	174.79	58.66	248
CH Plants	8.74	4.35	0.05	89.30	10.70	85.45	3.86	38.95	187	169.16	61.05	265
SEM	0.773	1.680	0.090	2.034	2.034	2.838	1.453	1.786	7.0	6.290	1.786	10.2
P value	0.158	0.203	0.217	0.073	0.073	0.067	0.265	0.284	0.465	0.465	0.284	0.191

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BCP or RUP: rumen bypass or undegraded feed crude protein; EDCP: effective degraded crude protein.

Table 4.2. *In situ* DM parameters of canola meals and pellets: comparisons between companies, and countries.

Items	Kd (%/h)	Fr (%)	T0 (h)	Residue at 0h (%)	S (%)	D (%)	U (%)	%BDM (%RUDM)	RUDM (g/kg DM)	%EDDM	EDDM (g/kg DM)
Canadian processing plants											
Plant 1 (M)	9.17	21.54	1.18	77.74	22.26	61.00	16.75	40.91	409	59.09	590
Plant 2 (M)	7.40	24.78	0.67	77.29	22.72	58.33	19.22	45.33	453	54.67	546
Plant 3 (P)	8.00	18.77	0.00	82.88	17.12	67.53	15.61	44.54	445	55.46	554
Plant 4 (P)	6.75	20.86	0.31	77.70	22.30	61.46	16.24	45.63	456	54.37	543
Plant 5 (P)	6.91	18.34	0.00	83.62	16.39	68.49	15.39	47.21	472	52.79	527
SEM	1.630	2.704	0.332	1.619	1.619	1.266	2.333	1.882	1.8	1.882	18.8
P value	0.680	0.580	0.237	0.271	0.271	0.072	0.780	0.301	0.301	0.301	0.301
Meals vs. Pellets											
SEM	7.899	13.106	1.607	5.124	5.124	6.136	11.310	9.123	9.1	9.123	91.2
P value	0.505	0.221	0.092	0.135	0.135	0.026	0.357	0.221	0.221	0.221	0.221
Chinese processing plants											
Plant A (M)	9.03	18.29	0.31	82.10	17.90	67.45	15.28	41.92	419	58.08	580
Plant B (M)	8.33	17.38	0.00	84.51	15.49	69.49	14.40	43.80	438	56.20	561
Plant C (M)	9.47	19.42	0.67	78.98	21.02	63.65	15.33	40.07	400	59.93	599
Plant D (M)	9.66	19.42	0.80	78.75	21.26	63.44	15.30	39.78	397	60.22	602
Plant E (M)	8.27	17.03	0.30	80.36	19.65	66.67	13.69	41.72	417	58.28	582
SEM	1.163	2.582	0.324	1.688	1.688	2.065	2.359	1.165	11.7	1.165	11.7
P value	0.732	0.923	0.403	0.232	0.232	0.430	0.977	0.432	0.432	0.432	0.432
Overall											
CA Plants	8.58	22.36	1.01	77.67	22.33	60.34	17.39	42.37	423	57.63	576
CH Plants	9.02	18.43	0.48	80.35	19.65	65.56	14.79	41.11	411	58.89	588
SEM	0.599	1.400	0.203	1.185	1.185	1.742	1.042	1.143	11.4	1.143	11.4
P value	0.548	0.013	0.055	0.087	0.087	0.016	0.025	0.360	0.360	0.360	0.360

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

Table 4.3. *In situ* CP parameters of canola seeds: comparisons between companies, and countries.

Items	Kd (%/h)	Fr (%)	Residue at 0h (%)	S (%)	D (%)	U (%)	%BCP (%RUP)	BCP (g/kg DM)	%RUP	%EDCP	EDCP (g/kg DM)
Canadian processing plants											
Plant 1 (M)	13.65	8.04	64.50	35.51	59.31[a]	5.19	23.30	59	53.31	76.71	175
Plant 2 (M)	13.20	8.49	58.35	41.65	51.38[ab]	4.95	21.66	53	48.03	78.34	177
Plant 3 (P)	14.60	6.04	62.52	37.48	56.73[ab]	3.78	20.90	52	46.85	79.10	180
Plant 4 (P)	27.30	15.21	66.58	33.42	56.41[ab]	10.17	21.06	52	47.32	78.95	177
Plant 5 (P)	22.60	12.10	58.69	41.31	49.57[b]	7.11	17.89	43	39.43	82.11	185
SEM	13.858	4.196	2.619	2.619	2.041	2.965	1.522	2.4	2.120	1.522	3.5
P value	0.640	0.507	0.299	0.299	0.045	0.515	0.340	0.187	0.187	0.340	0.432
Chinese processing plants											
Plant A (M)	18.50	8.55	66.15	33.85	59.68	5.58	20.19	50	45.87	79.81	181
Plant B (M)	18.20	9.99	57.86	42.14	52.88	5.87	18.98	46	41.94	81.02	179
Plant C (M)	19.15	11.28	63.80	36.20	56.67	7.14	20.72	52	47.18	79.28	180
Plant D (M)	16.20	9.36	63.60	36.40	57.67	5.94	21.53	53	48.39	78.47	176
Plant E (M)	5330.95	18.25	67.03	32.97	54.41	12.63	20.13	50	45.16	79.88	179
SEM	4339.720	6.002	6.778	6.778	3.695	4.762	1.578	4.0	3.629	1.578	3.5
P value	0.710	0.616	0.964	0.964	0.676	0.644	0.748	0.687	0.687	0.748	0.749
Overall											
CA Plants	48.00	10.90	63.14	36.86	56.45	7.02	21.34	53	48.42	78.66	178
CH Plants	1346.16	12.04	64.11	35.89	56.25	7.86	20.49	51	46.16	79.51	179
SEM	1240.79	2.496	1.860	1.860	1.268	1.902	0.608	1.6	1.461	0.608	1.3
P value	0.420	0.677	0.686	0.686	0.910	0.681	0.328	0.278	0.278	0.328	0.729

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BCP or RUP: rumen bypass or undegraded feed crude protein; EDCP: effective degraded crude protein.

Table 4.4. *In situ* DM parameters of canola seeds: comparisons between companies, and countries.

Items	Kd (%/h)	Fr (%)	T0 (h)	Residue at 0h (%)	S (%)	D (%)	U (%)	%BDM (%RUDM)	RUDM (g/kg DM)	%EDDM	EDDM (g/kg DM)
					Canadian processing plants						
Plant 1 (M)	7.92[b]	8.96	0.10	67.69	32.31	61.68	6.02	32.71	327	67.30	672
Plant 2 (M)	7.41[b]	9.44	0.64	61.39	38.61	56.46	5.89	31.22	312	68.78	687
Plant 3 (P)	9.86[b]	9.41	0.97	66.46	33.54	61.14	6.28	29.23	292	70.77	707
Plant 4 (P)	9.02[b]	12.17	0.27	71.53	28.48	62.79	8.74	33.83	338	66.18	661
Plant 5 (P)	15.19[a]	18.76	1.04	63.06	36.94	52.11	11.91	26.08	260	73.92	739
SEM	0.526	1.451	0.322	3.981	3.981	4.048	1.104	2.149	21.5	2.149	21.5
P value	0.017	0.053	0.395	0.456	0.456	0.441	0.115	0.296	0.295	0.296	0.295
					Chinese processing plants						
Plant A (M)	12.58	10.58	1.00	71.32	28.69	62.94	7.43	28.06[b]	280[b]	71.94[a]	719[a]
Plant B (M)	10.75	12.88	1.00	60.87	39.14	53.83	7.98	26.95 [b]	269[b]	73.05[a]	730[a]
Plant C (M)	10.23	12.06	0.54	70.19	29.82	61.75	8.44	31.27 [a]	312[a]	68.73[b]	687[b]
Plant D (M)	8.98	9.73	0.65	66.69	33.31	60.20	6.49	30.61 [a]	306[a]	69.39[b]	693[b]
Plant E (M)	12.83	17.19	0.42	69.93	30.07	57.70	12.23	31.36 [a]	313[a]	68.65[b]	686[b]
SEM	4.095	3.796	0.422	4.767	4.767	2.904	3.269	0.322	3.2	0.322	3.2
P value	0.957	0.489	0.724	0.843	0.843	0.727	0.571	0.005	0.005	0.005	0.005
					Overall						
CA Plants	9.25	10.83	0.74	67.05	32.95	59.81	7.35	31.37	313	68.63	686
CH Plants	10.93	12.68	0.65	68.22	31.77	59.51	8.72	30.19	301	69.82	698
SEM	0.978	1.687	0.143	1.608	1.608	1.493	1.296	0.927	9.3	0.927	9.3
P value	0.234	0.403	0.652	0.603	0.603	0.887	0.397	0.368	0.368	0.368	0.368

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

4.3.8. Hourly effective degradation of DM and CP during incubation of feedstocks and co-products from bio-oil processing: comparison among bio-oil processing plants and between two countries

The hourly effective degradation of CP from 0 to 48h is reported for canola meals and pellets (Table 4.5) and for canola seeds (Table 4.7). The degradation of CP was not different among Canadian plants for the first 12h ($P>0.05$) but were different after 24 and 48h ($P=0.036$ and $P=0.027$). A difference between mash and pellets started to be noticed following 8h of incubation ($P<0.05$). Plant 3 had the lowest effective degradation of CP starting at 2 hours of incubation (Table 4.5). On the other hand, similarities on the degradation of CP were no longer observed after 2 hours of incubation of Chinese meals. Starting at 4 hours of incubation, Plant C presented higher degradation than the other plants and the Plant B was the worst until 48 hours of incubation ($P<0.05$). Considering the performance of both countries, Chinese meals presented higher degradation of CP on 24 and 48h of incubation ($P=0.042$ and $P=0.040$, respectively).

All Canadian and Chinese samples performed similarly on the degradation of CP in canola seeds, except on the 2h incubation time-point where the Canadian companies showed some differences among themselves ($P=0.018$). There were no differences between the countries on the degradation of CP of canola seeds ($P>0.05$) (Table 4.7).

At 0h the effective degradation of DM of canola meals was different between Canada and China ($P=0.016$), when Canada had higher values than China (Table 4.6). But throughout the rest of the incubation study, no differences were observed between the two countries in any of the incubation times ($P>0.05$). Also, there were no differences on the meals within Canadian companies ($P>0.05$) or within Chinese companies ($P>0.05$). Moreover, the hourly effective degradation of DM of canola seeds showed neither differences within each country nor between countries ($P>0.05$) (Table 4.8).

Table 4.5. Hourly effective degradation of CP of canola meals and pellets: comparison between companies and countries.

Item	Hourly effective degradation (g/kg DM)						
	0h	2h	4h	8h	12h	24h	48h
				Canadian processing plants			
Plant 1 (M)	9	17	23	31	35	40 [a]	41 [a]
Plant 2 (M)	12	17	21	26	30	34 [ab]	36 [a]
Plant 3 (P)	7	8	8	8	9	9 [b]	9 [b]
Plant 4 (P)	8	14	18	25	29	34 [a]	35 [a]
Plant 5 (P)	5	11	14	22	26	31 [ab]	33 [ab]
SEM	1.4	2.0	2.4	2.7	2.7	2.5	2.2
P value	0.239	0.175	0.121	0.078	0.058	0.036	0.027
				Meals vs. Pellets			
SEM	6.9	9.7	11.4	12.9	13.1	11.9	10.7
P value	0.778	0.061	0.053	0.043	0.036	0.025	0.019
				Chinese processing plants			
Plant A (M)	5	15	21 [ab]	30 [ab]	35 [ab]	40 [ab]	41 [ab]
Plant B (M)	4	12	18 [b]	27 [b]	32 [b]	37 [b]	39 [b]
Plant C (M)	10	19	25 [a]	34 [a]	38 [a]	43 [a]	44 [a]
Plant D (M)	9	19	26 [a]	35 [a]	39 [a]	44 [a]	45 [a]
Plant E (M)	5	13	19 [b]	28 [b]	32 [b]	38 [b]	39 [b]
SEM	1.4	0.9	0.7	0.9	0.8	0.8	0.6
P value	0.091	0.056	0.006	0.011	0.010	0.041	0.032
				Overall			
CA Plants	7	14	19	27	31	36	37
CH Plants	7	16	23	31	36	41	42
SEM	1.3	1.6	1.8	2.0	2.0	1.9	1.7
P value	0.768	0.398	0.185	0.086	0.060	0.042	0.040

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

Deacon et al. (1988) presented the DM and CP disappearance of canola seeds after 0, 2, 4, 8, 12 and 24h of rumen incubation. The disappearance of DM of canola seeds reported by them was like ours from 0 to 8h of incubation (theirs: 29.8, 41.0, 46.9, and 58.4%; ours: 30 40, 47, and 58g/kg DM). But for 12 and 24h of incubation our disappearance was lower than Deacon et al. (64.84 vs. 78.2%; and 72.45 vs. 93.1%). On the other hand, the disappearance of CP was completely different between our studies with our values being inferior to the ones from Deacon et al. for all incubation times and the differences increase with longer incubation periods (0h: 13.1 vs. 35.3%; 2h: 19.9 vs. 49.1%; 23.2 vs. 69.8%; 26.6 vs. 70.6%; 4h: 23.2 vs. 69.8%; 8h: 26.6 vs. 70.6%; 12h: 28.0 vs. 87.1; 24h: 28.9 vs. 100.1%). Perhaps the huge difference observed on the CP values can be partially related to them not applying the NLIN procedure at SAS to adjust their incubation results since this study results are non-linear.

Table 4.6. Hourly effective degradation of DM of canola meals and pellets: comparison between companies and countries.

Item	Hourly effective degradation (g/kg DM)						
	0h	2h	4h	8h	12h	24h	48h
				Canadian processing plants			
Plant 1 (M)	20	29	36	46	53	61	63
Plant 2 (M)	20	27	33	42	48	56	59
Plant 3 (P)	15	24	31	41	48	58	61
Plant 4 (P)	20	26	32	40	46	55	59
Plant 5 (P)	21	28	34	43	49	58	62
SEM	1.3	0.7	1.5	2.6	2.9	2.4	1.3
P value	0.265	0.090	0.285	0.434	0.461	0.411	0.236
				Meals vs. Pellets			
SEM	4.2	3.4	7.5	12.4	14.0	11.6	6.3
P value	0.274	0.066	0.194	0.340	0.408	0.497	0.543
				Chinese processing plants			
Plant A (M)	15	25	32	43	50	59	61
Plant B (M)	13	23	30	41	48	57	61
Plant C (M)	18	28	35	46	52	60	63
Plant D (M)	19	28	36	47	53	61	64
Plant E (M)	17	26	33	43	50	59	62
SEM	1.5	1.0	1.1	1.4	1.5	1.2	0.7
P value	0.219	0.057	0.067	0.145	0.185	0.446	0.244
				Overall			
CA Plants	20	28	34	43	49	58	62
CH Plants	17	26	34	44	51	60	62
SEM	0.9	1.0	1.2	1.5	1.5	1.3	0.9
P value	0.016	0.222	0.797	0.593	0.423	0.351	0.503

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

Table 4.7. Hourly effective degradation of CP of canola seeds: comparison between companies and countries.

Item	Hourly effective degradation (g/kg DM)						
	0h	2h	4h	8h	12h	24h	48h
				Canadian processing plants			
Plant 1	13	17[c]	21	24	26	27	28
Plant 2	15	20[b]	22	25	27	28	28
Plant 3	13	19[b]	22	25	27	28	28
Plant 4	12	20[b]	24	28	29	30	30
Plant 5	14	22[a]	25	28	29	29	29
SEM	1.1	0.7	0.5	1.1	2.0	2.9	3.0
P value	0.346	0.018	0.078	0.244	0.623	0.862	0.879
				Chinese processing plants			
Plant A	12	19	22	26	28	28	29
Plant B	14	20	23	27	28	29	29
Plant C	11	18	22	25	27	28	28
Plant D	13	18	21	25	27	28	28
Plant E	11	24	25	27	28	28	28
SEM	1.7	5.8	2.8	1.4	0.9	0.5	0.5
P value	0.611	0.952	0.733	0.749	0.689	0.548	0.541
				Overall			
CA Plants	13	19	22	26	28	29	29
CH Plants	12	20	23	26	27	28	28
SEM	0.7	1.3	0.8	0.6	0.5	0.6	0.6
P value	0.648	0.547	0.629	0.960	0.738	0.470	0.430

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

Table 4.8. Hourly effective degradation of DM of canola seeds: comparison between companies and countries.

Item	Hourly effective degradation (g/kg DM)						
	0h	2h	4h	8h	12h	24h	48h
				Canadian processing plants			
Plant 1	30	38	45	55	62	71	74
Plant 2	36	43	49	57	63	71	75
Plant 3	31	41	48	59	66	74	77
Plant 4	26	35	43	54	61	72	76
Plant 5	34	46	55	65	71	75	76
SEM	3.7	3.4	3.3	3.2	3.5	5.1	6.9
P value	0.400	0.335	0.296	0.315	0.444	0.936	0.995
				Chinese processing plants			
Plant A	27	39	49	61	68	74	75
Plant B	32	41	49	59	65	72	74
Plant C	25	37	45	56	63	72	74
Plant D	31	40	47	57	64	72	75
Plant E	27	39	47	58	64	70	71
SEM	4.2	2.6	2.9	2.6	2.4	1.5	1.7
P value	0.664	0.662	0.945	0.896	0.880	0.365	0.359
				Overall			
CA Plants	30	40	47	57	64	72	75
CH Plants	29	40	47	58	64	72	74
SEM	1.6	1.3	1.3	1.3	1.2	1.2	1.5
P value	0.520	0.994	0.688	0.519	0.588	0.682	0.314

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

4.3.9. Intestinal digestion of undegraded protein and total tract digestion of protein of feedstocks and co-products from bio-oil processing: comparison among bio-oil processing plants and between two countries

The intestinal *in vitro* digestibility of DM and CP parameters on canola meals and pellets and canola seeds are displayed in Table 4.9 and Table 4.10. This study showed that the various samples of canola meals and pellets from the Canadian companies appeared to be similar for all parameters reported (P>0.05) and no differences were observed between meals mash and pelleted (P>0.05). The Chinese companies were also all similar (P>0.05). While the TDDM was higher in Chinese meals (P=0.018; 83.76 vs. 81.53%), the intestinal digestibility of proteins was higher in Canadian meals (P=0.016; 68.51 vs. 65.28%) (Table 4.9). Hvelplund and Madsen reported a value of 63% for the digestibility of N for rapeseed meals in 1993. This reported value is a bit lower than the one found for canola meals in this study that averaged 66.9% (IDP).

McKinnon, Olubobokun, Mustafa, Cohen, and Christensen (1995) found the total tract disappearance of DM and CP of canola meals to be 82.3 and 93%, respectively. From Table 4.9 we calculate the average of TDDM as 82.65% and TDP as 87.61%. The values for DM are similar between the two studies. But for CP we see that our study was lower than reported by McKinnon et al. of 93%. The difference could be possible due to the use of fecal residue in the study of McKinnon et al. (1995) to determine the total tract disappearance, while we just used the *in vitro* study.

When the intestinal digestibility of DM and CP of canola seeds were studied (Table 4.10), all the parameters analyzed regarding DM or CP were similar among the Canadian companies, the Chinese companies and between the two countries (P>0.05). Hvelplund, Weisbjerg, and Andersen (1992) when studying the degradability of proteins and its consequence on the intestinal digestibility, concluded that the digestibility of each feed was influenced by their degradability. This conclusion supports our results because the effective degradability of dry matter and crude protein on canola seeds (Table 4.7 and Table 4.8) were not different between countries, consequently the digestibilities of DM and CP were also similar (Table 4.10).

Table 4.9. *In vitro* DM and CP parameters of canola meals and pellets: comparisons between companies, and countries.

Item	%dBDM	%IDBDM	IDBDM (g/kg DM)	%TDDM	TDDM (g/kg DM)	%dIDP	IADP (g/kg DM)	IADP (g/kg CP)	TDP (g/kg DM)	TDP (g/kg CP)	%IADP	%TDP
Canadian companies												
Plant 1 (M)	43.63	13.52	135	82.63	826	68.38	106	247.53	379	885	24.75	88.51
Plant 2 (M)	40.38	14.06	140	79.23	792	69.30	121	292.38	357	867	29.24	86.74
Plant 3 (P)	53.22	19.00	190	83.29	832	73.79	123	293.83	372	892	29.38	89.30
Plant 4 (P)	50.20	18.92	189	81.82	818	72.98	132	318.13	366	882	31.81	88.24
Plant 5 (P)	62.54	24.43	244	85.37	853	70.87	140	330.68	361	860	33.07	88.08
SEM	8.318	5.025	50.2	0.993	9.9	2.990	17.6	53.587	11.0	15.2	5.353	1.522
P value	0.498	0.565	0.565	0.168	0.168	0.465	0.484	0.529	0.581	0.614	0.529	0.615
Meals vs. Pellets												
SEM	40.325	24.357	243.5	4.815	48.1	9.506	54.0	157.04	53.5	73.7	15.691	7.377
P value	0.186	0.227	0.565	0.086	0.085	0.255	0.291	0.336	0.872	0.863	0.336	0.860
Chinese processing plants												
Plant A (M)	50.12	16.47	164	83.64	836	65.36	104	241.08	378	870	24.11	87.09
Plant B (M)	54.32	19.27	192	83.77	837	67.58	120	280.33	372	866	28.03	86.68
Plant C (M)	46.66	14.40	143	83.60	835	65.29	95	218.88	384	883	21.89	88.36
Plant D (M)	45.02	13.82	138	83.42	834	64.86	96	217.33	393	882	21.74	88.27
Plant E (M)	53.72	18.20	181	84.32	843	64.48	110	260.65	364	856	26.07	85.66
SEM	5.710	2.574	25.8	1.131	11.4	1.528	9.7	19.700	5.3	7.4	1.969	0.737
P value	0.563	0.424	0.424	0.980	0.980	0.622	0.358	0.209	0.145	0.267	0.209	0.265
Overall												
CA Plants	42.54	13.70	136	81.53	815	68.51	110	262.35	379	879	26.23	87.92
CH Plants	49.40	16.07	160	83.76	837	65.28	103	239.39	372	872	23.94	87.29
SEM	2.974	1.487	14.9	0.681	6.8	0.938	6.2	15.964	7.3	7.7	1.596	0.771
P value	0.081	0.207	0.207	0.018	0.018	0.016	0.363	0.251	0.423	0.502	0.252	0.504

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. Multi-treatment comparisons using Tukey method CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals; dBDM: digestibility of rumen bypass dry matter; IDBDM: (intestinal digestible rumen bypass DM; TDDM: total digestible DM; IDP: intestinal digestibility of protein; IADP: intestinally absorbable feed protein; TDP: total digestible protein.

Table 4.10. *In vitro* DM and CP parameters of canola seeds: comparisons between companies, and countries.

Item	%dBDM	%IDBDM	IDBDM (g/kg DM)	%TDDM	TDDM (g/kg DM)	%dIDP	IADP (g/kg DM)	IADP (g/kg CP)	TDP (g/kg DM)	TDP (g/kg CP)	%IADP	%TDP
Canadian companies												
Plant 1	66.43	19.53	195	90.23	902	54.55	24	107	208	910	10.77	91.04
Plant 2	67.07	19.17	191	91.05	910	54.72	21	94	208	922	9.40	92.26
Plant 3	63.96	16.59	165	91.05	910	43.13	16	72	205	901	7.26	90.15
Plant 4	64.03	18.33	183	89.70	897	47.66	14	62	209	934	6.24	93.46
Plant 5	42.39	7.607	76	89.71	897	35.69	8	36	208	924	3.69	92.49
SEM	2.430	3.360	33.6	0.397	4.0	6.558	5.2	21.9	1.4	10.2	2.189	1.027
P value	0.097	0.331	0.331	0.237	0.238	0.376	0.332	0.320	0.399	0.311	0.320	0.313
Chinese processing plants												
Plant A	59.03	13.65	136	90.91	909	44.20	16	70	206	910	7.06	91.03
Plant B	55.02	12.01	120	89.79	897	32.80	10	48	199	902	4.81	90.20
Plant C	63.20	16.44	164	90.43	904	43.47	15	67	208	914	6.75	91.42
Plant D	65.96	17.65	176	90.89	908	46.45	18	80	204	907	8.02	90.78
Plant E	58.80	13.66	136	90.65	906	47.20	8	37	213	951	3.71	95.15
SEM	3.937	2.333	23.3	0.496	5.0	8.345	7.3	32.30	7.9	38.7	3.228	3.871
P value	0.339	0.388	0.388	0.532	0.536	0.922	0.721	0.726	0.662	0.766	0.725	0.765
Overall												
CA Plants	62.76	17.22	172	90.24	902	48.99	18	80	208	920	8.09	92.03
CH Plants	61.24	15.14	151	90.58	905	43.90	13	61	207	919	6.10	91.99
SEM	2.556	1.337	13.4	0.212	2.1	2.555	2.9	12.7	2.0	12.029	1.269	1.203
P value	0.672	0.277	0.277	0.262	0.263	0.170	0.214	0.222	0.664	0.9788	0.222	0.979

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. Multi-treatment comparisons using Tukey method. CA: Canada; CH: China; dBDM: digestibility of rumen bypass dry matter; IDBDM: (intestinal digestible rumen bypass DM; TDDM: total digestible DM; IDP: intestinal digestibility of protein; IADP: intestinally absorbable feed protein; TDP: total digestible protein.

4.3.10. Truly absorbable nutrient supply to dairy cows and feed milk values (FMV) of feedstocks and co-products from bio-oil processing: comparison among bio-oil processing plants and between two countries

The prediction of nutrient supply to dairy cows using the DVE/OEB system is introduced in Table 4.11 for canola meals and pellets and in Table 4.12 for canola seeds. Nutrient supply was similar among the Canadian companies analyzed (P>0.05) and no difference was detected between canola meals mash and pellets (P>0.05). The Chinese meals, however, presented a difference on the content of microbial protein synthesized in the rumen based on the available rumen degradable protein (MREN) (P=0.035). When comparing between countries, the microbial protein synthesized in the rumen based on the available energy (MREE) (P=0.0.11; 86 vs. 80 g/kg DM) and the rumen synthesized microbial protein digested in the small intestine (DVME) (P=0.011; 55 vs. 51 g/kg DM) were higher in the Chinese meals.

The results from the DVE/OEB system applied on canola seeds from Canada and China are presented in Table 4.12, where we can see that only the truly digested protein in the small intestine (DVE) was different among the seeds from Canadian companies (P=0.007) and no other differences were observed among the companies in Canada (P>0.05) or in China (P>0.05), and between the countries (P>0.05).

The values of MREE, DVME, and BCP reported by Theodoridou and Yu (2013) for canola meals (*B. napus*) were similar to ours. The canola meals from our study, however, presented higher MREN and OEB (difference between the potential MREN and MREE) values, and consequently lower DVBE (truly bypass feed crude protein absorbed in the small intestine), and DVE values than theirs.

Table 4.11. Truly absorbed nutrient supply of canola meals and pellets revealed with the DVE/OEB system: comparisons between companies, and countries.

Items	BCP	MREE	MREN	DVME	DVBE	DVE	OEB
				(g/kg DM)			
			Canadian processing plants				
Plant 1 (M)	188	82	240	52	128	167	157
Plant 2 (M)	205	74	206	47	143	175	131
Plant 3 (P)	201	76	215	48	149	186	138
Plant 4 (P)	215	76	199	48	157	191	123
Plant 5 (P)	230	81	190	52.02	164	205	108
SEM	11.5	2.2	16.8	1.4	10.9	9.0	14.8
P value	0.289	0.232	0.340	0.232	0.307	0.254	0.321
			Meal vs Pellet				
SEM	55.5	10.7	81.3	6.8	35.1	29.1	71.6
P value	0.178	0.806	0.250	0.808	0.170	0.133	0.217
			Chinese processing plants				
Plant A (M)	193	84	242^{ab}	53	126	166	159^{b}
Plant B (M)	203	84	224^{b}	54	137	178	138^{c}
Plant C (M)	175	86	260^{a}	55	114	156	173^{a}
Plant D (M)	179	88	265^{a}	56	116	159	177^{a}
Plant E (M)	197	86	227^{b}	54	127	169	141^{c}
SEM	7.5	1.3	4.1	0.9	7.4	7.1	2.5
P value	0.138	0.237	0.035	0.237	0.265	0.279	0.010
			Overall				
CA Plants	194	80	229	51	132	169	149
CH Plants	187	86	246	55	122	164	160
SEM	7.0	1.7	10.9	1.1	5.3	4.7	9.7
P value	0.465	0.011	0.201	0.011	0.136	0.444	0.352

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on the energy available; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance.

Table 4.12. Truly absorbed nutrient supply of canola seeds revealed with the DVE/OEB system: comparisons between companies, and countries.

Items	BCP	MREE	MREN	DVME	DVBE	DVE	OEB
				(g/kg DM)			
Canadian processing plants							
Plant 1	59	59	169	38	32	62[a]	109
Plant 2	53	71	171	45	28	65[a]	99
Plant 3	52	58	175	37	21	50[c]	116
Plant 4	52	60	172	38	25	55[b]	111
Plant 5	43	58	180	37	14	42[d]	121
SEM	2.4	4.6	3.6	2.9	2.9	3.6	6.0
P value	0.187	0.368	0.385	0.367	0.136	0.007	0.370
Chinese processing plants							
Plant A	50	55	176	36	22	50	120
Plant B	46	53	174	33	15	40	121
Plant C	52	62	175	40	22	55	112
Plant D	53	58	171	37	24	54	112
Plant E	50	56	174	36	23	52	117
SEM	4.0	7.2	3.9	4.6	3.4	6.0	6.3
P value	0.687	0.721	0.775	0.721	0.407	0.464	0.179
Overall							
CA Plants	53	62	173	39	26	57	110
CH Plants	51	58	173	37	22	51	115
SEM	1.6	3.2	1.4	2.0	1.9	2.7	3.9
P value	0.278	0.252	0.661	0.252	0.137	0.140	0.209

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; Overall: compares only meals. Multi-treatment comparisons using Tukey method. BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on the energy available; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance.

The nutrient supply of canola meals and seeds was also determined according to the NRC model and the results are brought in Table 4.13 and Table 4.14. The study on canola meals shows that the samples from Canadian companies were similar in all aspects analyzed ($P>0.05$) and that only the microbial protein synthesized in the rumen based on available rumen degradable protein (MCP RDP) was different among Chinese companies ($P=0.032$). and the comparison between countries showed that the amount of endogenous CP (ECP) and the truly absorbed ECP in the small intestine (AECP) are higher in the Canadian meals ($P=0.001$, for both).

Theodoridou and Yu (2013) reported similar results to ours for ECP, AECP, AMCP and MCP_{TDN}; lower than ours for MCP_{RDP} and DPB; and higher for RUP, ARUP, and MP. And Heim

and Krebbs (2020) on a review about canola meals fed to cattle, reported that the amount of RUP for canola meals ranged from 10.1 to 75%CP among the literature consulted. In our study, the RUP was around 17.2%DM, equivalent to 40.22%CP, which is inside that range. But Paz et al. 2014, using canola meal with 40.7%DM of CP, found an *in situ* RUP of 24.3%CP and an *in vitro* mean of 32.1%CP, which is lower than the RUP found in this study for canola meals. However different these results might be, they are all accepted because many factors can influence the canola meal such as species of canola seed, method of oil extraction etc.

The NRC model showed that the canola seeds from Canada were different on the ECP, and the rumen degraded protein balance (DPB) (P=0.001 and P=0.043, respectively). While the Chinese companies' seeds were only different on the amount of ECP (P=0.021). Although these differences within countries were observed, no differences were observed between countries for any of the aspects studied (P>0.05).

Table 4.13. Nutrient supply of canola meals and pellets revealed with NRC model: comparisons between companies, and countries.

Items	MCP_RDP	MCP_TDN	AMCP	RUP	ARUP	ECP	AECP	MP	DPB
				(g/kg DM)					
Canadian processing plants									
Plant 1 (M)	220	79	50	169	116	10	4	114	165
Plant 2 (M)	192	76	49	184	129	10	4	121	136
Plant 3 (P)	200	84	54	181	134	10	4	129	135
Plant 4 (P)	188	80	51	194	141	10	4	132	125
Plant 5 (P)	181	79	50	207	147	10	4	135	119
SEM	13.3	1.8	1.2	10.3	9.8	0.0	0.0	8.5	17.8
P value	0.3423	0.274	0.275	0.290	0.307	0.094	0.144	0.362	0.395
Meal vs Pellet									
SEM	64.5	8.7	5.6	50.1	31.6	0.1	0.1	26.5	86.6
P value	0.256	0.118	0.119	0.179	0.170	0.048	0.067	0.188	0.231
Canadian processing plants									
Plant A (M)	222^{ab}	81	52	173	113	10	4	113	166^{bc}
Plant B (M)	207^{b}	78	50	183	124	10	4	119	151^{c}
Plant C (M)	236^{a}	80	51	157	103	10	4	106	182^{ab}
Plant D (M)	241^{a}	79	50	161	104	10	4	107	189^{a}
Plant E (M)	210^{b}	76	48	178	114	10	4	112	157^{c}
SEM	3.1	0.9	0.5	6.8	6.7	0.0	0.0	4.3	2.7
P value	0.032	0.060	0.060	0.138	0.265	0.135	0.120	0.280	0.016
Overall									
CA Plants	211	78	50	174	119	10	4	116	156
CH Plants	225	79	50	169	110	10	4	110	172
SEM	8.7	1.1	0.7	6.3	4.8	0.1	0.0	3.0	9.5
P value	0.191	0.588	0.593	0.465	0.136	0.001	0.001	0.123	0.181

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN3x; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; ECP: endogenous protein in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance.

Table 4.14. Nutrient supply of canola seeds revealed with NRC model: comparisons between companies, and countries.

Items	MCP_RDP	MCP_TDN	AMCP	RUP	ARUP	ECP	MP	DPB
			(g/kg DM)					
Canadian processing plants								
Plant 1	149	150	96	53	29	11.^{ab}	87	-2^{b}
Plant 2	150	142	91	48	25	11^{a}	82	6^{a}
Plant 3	153	154	96	46	19	11^{b}	83	-3^{b}
Plant 4	150	149	95	47	22	10^{d}	82	1^{ab}
Plant 5	157	153	98	39	13	11^{c}	78	2^{ab}
SEM	2.1	3.7	2.4	2.1	2.6	0.0	2.3	1.7
P value	0.433	0.417	0.416	0.187	0.135	0.001	0.303	0.043
Chinese processing plants								
Plant A	154	158	101	45	20	10^{ab}	85	-7
Plant B	152	160	102	41	13	10^{ab}	79	-8
Plant C	153	150	96	47	20	11^{ab}	81	3
Plant D	149	155	99	48	22	11^{a}	84	-6
Plant E	152	156	99	45	21	10^{b}	84	-5
SEM	3.0	6.7	4.3	3.6	3.1	0.0	4.7	11.3
P value	0.749	0.658	0.658	0.687	0.407	0.021	0.949	0.916
Overall								
CA Plants	151	149	95	48	23	11	83	1
CH Plants	152	155	99	46	20	11	83	-4
SEM	1.1	3.3	2.1	1.5	1.7	0.1	1.8	3.5
P value	0.729	0.063	0.064	0.278	0.138	0.153	0.957	0.167

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method. MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN_{3x}; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; ECP: endogenous protein in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance.

Regardless of the method chosen to analyze the nutrient supply, Chinese meals presented variability on the amount of microbial protein synthesized in the rumen based on the rumen degraded feed protein (MREN in DVE) (Table 4.11) and (MRC_{RDP} in NRC) (Table 4.13).

Useful to the dairy production is also the prediction of milk production. Table 4.15 and Table 4.16 shows the feed milk value (FMV) for canola meals and seeds based on three systems: DVE/OEB (DVE value), NRC (MP value), and energy (NEL) of the feed. The FMV according to the DVE/OEB system for canola meals and pellets from Canadian companies (P=0.243), from Chinese companies (P=0.283), and between countries (P=0.443) seemed to be similar on this study. Similar behavior was observed on the FMV based on the NRC model, where Canadian (P=0.34) and Chinese (P=0.278) companies, and the comparison between countries (P=0.269) did

not result in any differences. But the FMV based on the energy showed differences among the Canadian plants (<0.001) with the pellets being higher in FMV than mash (P=0.005). The FMV energy of Chinese meals were similar (P>0.05). And the FMV energy of canola meals was the same between countries (P=0.269).

Table 4.15. Feed milk value (FMV) model parameters of canola meals and pellets: comparisons between companies, and countries.

Items	FMV_DVE	FMV_NRC	FMV_Energy
		(kg milk/kg DM feed)	
	Canadian companies		
Plant 1 (M)	3.40	3.48	2.62^{ab}
Plant 2 (M)	3.58	3.70	2.54^{c}
Plant 3 (P)	3.79	3.91	2.67^{a}
Plant 4 (P)	3.90	4.02	2.60^{bc}
Plant 5 (P)	4.18	4.11	2.60^{bc}
SEM	0.177	0.266	0.022
P value	0.243	0.374	<0.001
	Meals vs. Pellets		
SEM	0.566	0.825	0.080
P value	0.127	0.196	0.005
	Chinese companies		
Plant A (M)	3.38	3.45	2.64
Plant B (M)	3.63	3.62	2.62
Plant C (M)	3.18	3.22	2.65
Plant D (M)	3.24	3.25	2.65
Plant E (M)	3.45	3.41	2.60
SEM	0.147	0.129	0.016
P value	0.283	0.278	0.053
	Overall		
CA Plants	3.43	3.54	2.61
CH Plants	3.34	3.35	2.63
SEM	0.100	0.090	0.015
P value	0.443	0.112	0.269

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

The FMV of canola seeds from the Canadian companies were different only when evaluated based on the DVE/OEB system (P=0.019) but was not different from the Chinese companies' seeds (P=0.138). The other models (NRC and NEL Energy) did not result in

differences among the companies within each country or between the two countries' companies (P>0.05).

Table 4.16. Feed milk value (FMV) model parameters of canola seeds: comparisons between companies, and countries.

Items	FMV_DVE	FMV_NRC	FMV_Energy
		(kg milk/kg DM feed)	
		Canadian companies	
Plant 1	1.27[a]	2.64	4.62
Plant 2	1.33[a]	2.51	4.52
Plant 3	1.03[bc]	2.53	4.76
Plant 4	1.12[b]	2.49	4.63
Plant 5	0.86[c]	2.39	4.66
SEM	0.073	0.066	0.073
P value	0.019	0.282	0.150
		Chinese companies	
Plant A	1.03	2.60	4.68
Plant B	0.82	2.41	4.80
Plant C	1.12	2.46	4.65
Plant D	1.12	2.57	4.67
Plant E	1.06	2.55	4.71
SEM	0.120	0.139	0.086
P value	0.485	0.938	0.449
		Overall	
CA Plants	1.17	2.52	4.63
CH Plants	1.05	2.52	4.70
SEM	0.055	0.053	0.041
P value	0.138	0.953	0.175

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals. Multi-treatment comparisons using Tukey method.

4.5. Chapter summary

The *in situ* parameters analyzed for DM and CP on canola meals and pellets and canola seeds, showed only minor differences. U (P=0.025) was higher in Canadian meals, and D was higher (P=0.016) in Chinese meals. The hourly degradation studies of DM and CP showed no differences between countries, except the hourly degradation of CP that were higher in Chinese

canola meals on 24 (P=0.042) and 48 hours (P=0.040) of incubation. The *in vitro* intestinal digestibility of DM and CP showed that the percentage of TDDM and dIDP of the canola meals from China were higher (P=0.018 and P=0.0.16, respectively) than from Canada. The DVE/OEB system showed that canola meals from China had higher amounts of MREE and DVME (P=0.011 and P=0.011). And the NRC model showed that canola meals from Canada had higher contents of ECP and AECP than China (P=0.001 and P=0.001). The nutrient supply prediction using both the DVE/OEB or the NRC system showed similar results for canola meals. The FMV from the three systems showed no differences between countries and averaged: FMV_{DVE}: 3.39; FMV_{NRC}: 3.45; FMV_{Energy}: 2.62 for canola meals, and FMV_{DVE}: 1.11; FMV_{NRC}: 2.52; FMV_{Energy}: 4.67 for canola seeds. In conclusion, the *in vitro* intestinal study and the nutrient supply systems were not able to identify many differences between the samples of canola meals or seeds from Canada or China.

5. REVEAL INTRINSIC MOLECULAR STRUCTURES FROM FEEDSTOCKS AND CO-PRODUCTS FROM CANOLA BIO-OIL PROCESSING USING ADVANCED VIBRATIONAL MOLECULAR SPECTROSCOPY TECHNIQUES

5.1. Abstract

Canola is an oilseed widely produced in Canada. The oil extracted is designated to human consumption and the co-product, the meal, that is rich in protein is directed for animal consumption. Different crops and processing methods are known to cause changes on the composition of feedstuffs. Also, the molecular structures of proteins can affect its availability to gastrointestinal enzymes impacting its digestibility and absorption. ATR-FTIR is technique that measures the absorbance of infrared light on the infrared region and through imaging techniques we can identify and quantify molecules and functional groups present in a matter. Therefore, the aim of this study was to identify the carbohydrate and protein-relates structures on canola seeds and meals from different crushing companies in Canada and in China. Samples were obtained from five different companies in each country and analyzed at the University of Saskatchewan. The procedure MIXED at SAS 9.4 was used and significance was declared when $P<0.05$. Multiple comparisons were through the Tukey method. As results, differences were observed on all total carbohydrates, structural carbohydrates, and cellulosic compounds ($P<0.05$), except TC2 and STC1 ($P>0.05$) of canola meals, where Chinese meals presented higher peaks of these structures than the Canadian meals. Similarly, the carbohydrate-related structure of canola seeds where different between countries except for STC3 height, CEC and STC areas ($P>0.05$). The protein-related structures were similar for the canola seeds from both countries. However, Chinese meals presented higher peaks of amide I, α-helix, and β-sheet heights, α-helix:β-sheet ratio, total amide and amide I areas ($P<0.05$). The principal component analysis was able to explain over 93% of the

variabilities in the carbohydrate and protein structures samples and was not able to separate the samples from the two countries.

5.2. Introduction

Canola (*Brassica napus*) has been extensively produced in Canada since its development in 1970s. Canola was developed due the necessity for an oilseed with high amounts of oil that could be extracted for human consumption that was palatable (low erucic acid levels) and the co-product could be utilized to minimize waste (low glucosinolates levels increases palatability of the meal for animals consumption). Therefore, canola is the oil rapeseed that resulted from extensive studies of plant breeding and selection at the University of Manitoba by Dr. Stefansson and his team in 1974 (Rapeseed Association of Canada, 1974).

Regular wet laboratory analyses determine the chemical composition of feedstuffs but fail to characterize their carbohydrate and protein structures, meaning they do not provide information related to the real nutrient supply and utilization that are essential for animal performance (Ban, Prates, Feng, Khan, and Yu, 2021). Plus, the use of harsh chemicals for wet chemistry analyses can alter and destroy these structures consequently over or underestimating results (Chen, Zhang, and Yu, 2014).

Spectroscopy studies the interaction of light and matter and provides information on the chemical composition and physical structures at specific locations of a sample through imaging techniques (Wang, Yao and Parthasarathy, 2008; Ling, Qi, Shao, and Chen, 2015). A quick and non-invasive method of analysis that observes the mid-infrared region (ca. 4000 to 800 cm^{-1}) called attenuated total reflectance Fourier transform infrared vibrational spectroscopy (ATR-FTIR) identifies molecules and functional groups based on their infrared light absorbance on this region (Ban, Prates and Yu, 2017).

The structure of protein in a matter is essential to gain knowledge about its availability to the animals. For instance, protein is stored in seeds as cruciferin or napin. Perera, McIntosh and Wanasundara (2016) mentioned that 60% of *B. napus*'s protein storage is as cruciferin and only 20% is as napin. The napin fraction contains 40-46% of α-helix and lower amounts of β-sheet,

while the cruciferin contains about 10% of α-helix and around 50% of β-sheet. High amounts of β-sheet indicate a low protein value because this structure provides low availability to the gastrointestinal enzymes (Theodoridou and Yu, 2013; Theodoridou, Vail, and Yu, 2014). Therefore, identifying the presence of these structures on feedstuffs is essential to understand how the animal can respond when fed.

The use of ATR-FTIR to characterize the carbohydrate and protein structures in feedstuffs is important to provide data to increase the knowledge of these structures on these materials and to identify structural variations due to transport and processing methods. Furthermore, the aim of this study was on the identification of the intrinsic carbohydrate and protein structures of canola seeds and meals from five crushing companies in Canada and five in China.

5.3. Materials and Methods

5.3.1. Sampling and analyses

The samples of feedstocks and co-products from bio-oil processing were collected from Canada and China by the Canola Council of Canada in 2016. Samples were collected from five crusher companies operating in China and five in Canada. The companies crushed seeds imported from Canada. Three of the five Canadian crushers samples of meals were pelleted and two were mash, like China's meals that were all mash. Samples of seeds and meals were collected from different batches from each crusher, stored and transported to the University of Saskatchewan in Canada for further analyses. The samples were provided by each company's quality control laboratory and are to be considered representative of the reality of those crushers.

5.3.2. Attenuated Total Reflectance (ATR) - Fourier Transform Infrared (FTIR) Vibrational Molecular Spectroscopy

The spectral analyses of the intrinsic molecular structures of protein and carbohydrate of the canola seeds, meals and pellets were obtained at the University of Saskatchewan, using the FTIR- ATR vibrational spectroscopy model 4200 (JASCO Corporation, Tokyo, Japan) machine at the mid-infrared spectrum (ca. 4000 to 800 cm^{-1}). With the assistance of the OMNIC 7.3 software (Spectra Tech., Madison, WI, USA), the spectra was represented in images, and transcribed into numbers, so they later could be processed by Unscrambler X 10.3 (CAMO Software, 2013) for the multivariate analyses.

5.3.3. Statistical analysis

Fitting a Complete Randomized Block Design (RCBD), with country and company as fixed effects and batch as random effect, the procedure MIXED was used on SAS® 9.4 (SAS Institute, USA).

$$y = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

Where, μ= overall mean; τ_i= fixed effect; β = random effect; ϵ_{ij}= error.

$\beta_j \sim$ NIID (Normally, Identically, and Independently distributed)

$\epsilon_{ij} \sim$ NIID (Normally, Identically, and Independently distributed)

When $P<0.05$ results were considered significant. The multiple comparison was tested through the Tukey method.

5.4. Results and Discussion

5.4.1. Molecular Structure Spectral Features Revealed with Vibrational Molecular Spectroscopy for Feedstock and Co-Products from Bio-Oil Processing

The intrinsic molecular carbohydrate structures of canola meals and seeds are shown in Table 5.1 and Table 5.2. This study analyzed the heights of four total carbohydrate peaks (TC1, TC2, TC3 and TC4) as well as the cellulosic compounds (CEC), and four of the structural carbohydrates (STC1, STC2, STC3, and STC4), and the areas of total carbohydrate, cellulosic compounds, and structural carbohydrates.

The analyses within Canada showed differences between samples that were pelleted and mash for most structures studied ($P<0.05$), except for STC1 and STC2 that presented differences among companies ($P<0.05$) but no differences between pellets or mash ($P>0.05$), and the area of CEC that was same among the five Canadian crushers ($P>0.05$) (Table 5.1). Amidst the Chinese crushers, only TC4 height varied ($P=0.040$). Interestingly for the CHO structures studied on this project, almost all were in higher concentration of the Chinese meals, except for TC2 and STC1 that were the same between both countries ($P=0.057$ and $P=0.700$, respectively).

The seeds were more even in general within and between countries than the meals (Table 5.2). There were no differences within the crushing companies in China ($P>0.05$), and only STC1 and STC3 showed some differences among the Canadian crushers ($P=0.009$ and $P=0.044$, respectively). Like the meals, most parameters were different between countries ($P<0.05$) apart from STC3 height ($P= 0.100$), and the areas of CEC ($P=0.804$) and STC ($P=0.284$). Opposite from the meals, the seeds presented higher carbohydrate structures concentrated on the seeds from Canadian crushers.

Table 5.1. Using FTIR-ATR molecular spectroscopic technique to determine carbohydrates-related molecular spectral features of canola meals and pellets: comparisons between companies, countries, and periods.

Items	TC1	TC2	TC3	TC4	CEC	STC1	STC2	STC3	STC4	TC	CEC	STC
	Height									Area		
Canadian processing plants												
Plant 1 (M)	0.48^{b}	0.46^{b}	0.35	0.16^{b}	0.06	0.06 ^{a}	0.06^{a}	0.11	0.06	76.18^{b}	3.80	18.24^{ab}
Plant 2 (M)	0.51^{ab}	0.49^{ab}	0.37	0.19^{ab}	0.06	0.03 ^{b}	0.05^{b}	0.11	0.12	81.56^{ab}	3.24	16.51^{b}
Plant 3 (P)	0.55^{a}	0.52^{a}	0.39	0.20^{a}	0.07	0.04 ^{ab}	0.06^{ab}	0.12	0.11	86.49^{a}	3.53	18.26 ^{ab}
Plant 4 (P)	0.51^{ab}	0.49^{ab}	0.38	0.19^{a}	0.07	0.04 ^{ab}	0.06^{a}	0.12	0.13	82.30^{ab}	3.51	18.58 ^{a}
Plant 5 (P)	0.51^{ab}	0.49^{ab}	0.37	0.18^{ab}	0.07	0.04 ^{ab}	0.06^{a}	0.11	0.13	80.94^{ab}	3.56	18.23^{ab}
SEM	0.011	0.010	0.010	0.010	0.003	0.005	0.002	0.005	0.019	2.010	0.141	0.460
P value	0.010	0.041	0.047	0.028	0.253	0.041	0.013	0.204	0.071	0.022	0.096	0.032
Meals vs. Pellets												
SEM	0.061	0.058	0.054	0.050	0.015	0.027	0.011	0.023	0.097	10.521	0.739	2.310
P value	0.027	0.041	0.016	0.023	0.038	0.324	0.052	0.028	0.047	0.023	0.974	0.022
Chinese processing plants												
Plant A	0.51	0.49	0.38	0.19^{b}	0.08	0.05	0.06	0.11	0.13	82.08	3.94	19.48
Plant B	0.52	0.49	0.38	0.19^{ab}	0.07	0.05	0.07	0.12	0.12	83.56	3.94	20.55
Plant C	0.52	0.49	0.38	0.20^{a}	0.07	0.04	0.06	0.12	0.12	84.48	3.82	19.13
Plant D	0.52	0.49	0.38	0.19^{ab}	0.07	0.05	0.07	0.12	0.12	83.61	3.86	19.98
Plant E	0.52	0.48	0.37	0.19^{ab}	0.07	0.05	0.07	0.12	0.12	82.79	3.77	19.88
SEM	0.006	0.007	0.004	0.003	0.002	0.003	0.003	0.002	0.003	0.828	0.150	0.472
P value	0.377	0.603	0.250	0.040	0.258	0.194	0.165	0.101	0.281	0.288	0.834	0.250
Overall												
CA Plants	0.50	0.47	0.35	0.17	0.06	0.05	0.06	0.11	0.08	78.57	3.56	17.47
CH Plants	0.52	0.49	0.38	0.19	0.07	0.05	0.06	0.12	0.12	83.35	3.83	19.73
SEM	0.007	0.007	0.006	0.006	0.002	0.004	0.002	0.003	0.011	1.182	0.121	0.403
P value	0.011	0.057	0.005	0.001	<0.001	0.700	<0.001	0.002	0.006	0.002	0.023	<0.001

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals; TC: total carbohydrate; STC: structural carbohydrate; CEC: cellulosic compound; 1, 2, 3 and 4: correspond to the different peaks.

Table 5.2. Using FTIR-ATR molecular spectroscopic technique to determine carbohydrates-related molecular spectral features of canola seeds: comparisons between companies, countries, and periods.

Items	TC1	TC2	TC3	TC4	CEC	STC1	STC2	STC3	STC4	TC	CEC	STC
					Height						Area	
					Canadian processing plants							
Plant 1	0.55	0.49	0.36	0.19	0.07	0.07^{b}	0.07	0.10^{b}	0.11	84.06	3.69	20.60
Plant 2	0.55	0.53	0.37	0.18	0.07	0.09^{ab}	0.08	0.12^{ab}	0.03	87.67	4.36	22.77
Plant 3	0.56	0.51	0.37	0.16	0.07	0.09^{ab}	0.08	0.12^{ab}	0.03	85.98	4.25	23.23
Plant 4	0.55	0.51	0.37	0.16	0.07	0.10^{a}	0.09	0.12^{ab}	0.04	84.26	4.27	24.49
Plant 5	0.53	0.51	0.37	0.16	0.07	0.10^{a}	0.09	0.13^{a}	0.02	83.20	4.35	24.94
SEM	0.244	0.214	0.019	0.018	0.005	0.008	0.005	0.007	0.033	3.906	0.246	1.281
P value	0.915	0.655	0.981	0.664	0.954	0.009	0.050	0.044	0.189	0.873	0.225	0.075
					Chinese processing plants							
Plant A	0.50	0.48	0.33	0.12	0.06	0.10	0.08	0.11	0.00	77.15	4.11	22.21
Plant B	0.51	0.48	0.34	0.14	0.06	0.10	0.09	0.12	0.00	78.63	4.10	22.86
Plant C	0.51	0.49	0.35	0.14	0.07	0.10	0.08	0.12	0.01	79.69	4.13	22.41
Plant D	0.49	0.48	0.33	0.13	0.07	0.10	0.08	0.12	0.00	76.94	4.29	22.44
Plant E	0.48	0.47	0.32	0.14	0.06	0.10	0.08	0.12	0.00	74.16	4.03	21.78
SEM	0.012	0.011	0.010	0.009	0.002	0.003	0.003	0.003	0.012	1.838	0.100	0.707
P value	0.414	0.503	0.283	0.531	0.582	0.747	0.939	0.547	0.657	0.221	0.377	0.800
						Overall						
CA Plants	0.55	0.51	0.37	0.17	0.07	0.09	0.08	0.12	0.05	84.81	4.16	23.08
CH Plants	0.50	0.48	0.34	0.14	0.06	0.10	0.08	0.12	0.00	77.59	4.14	22.40
SEM	0.010	0.010	0.009	0.007	0.001	0.003	0.002	0.003	0.011	1.737	0.083	0.592
P value	<0.001	0.005	<0.001	<0.001	<0.004	0.033	0.044	0.100	<0.001	<0.001	0.804	0.284

Means in the same column with different letters differ significantly (P < 0.05). SEM: standard error of the mean. CA: Canada; CH: China; TC: total carbohydrate; STC: structural carbohydrate; CEC: cellulosic compound; 1, 2, 3 and 4: correspond to the different peaks.

The inherent protein structures of canola meals and seeds are displayed in Table 5.3 and Table 5.4. Table 5.3 shows that only the heights of the α-helix and β-sheet were higher in the pelleted meals than the mash (P=0.028 and P=0.032, respectively). Similar to the carbohydrate structures, the protein structures of the canola meals from Chinese crusher were all different among themselves (P<0.05). Chinese samples presented higher Amide I height (P=0.011), α-helix height (P=0.001), β-sheet height (P=0.012), α-helix to β-sheet ratio (P=0.008), Amide area (P=0.038), and Amide I area (P=0.019) than the Canadian meals. Despite the variations observed on the protein structures of the meals, the seeds did not result in any differences between countries or within Chinese crushers (P>0.05). Only the α-helix to β-sheet ratio of Canadian seeds varied among companies (P<0.001).

Industry processing methods can affect protein structures (Theodoridou and Yu, 2013). It was observed that the α-helix:β-sheet ratio decreased from the canola seeds to canola meals (1.07 vs. 1.02, respective averages between countries), indicating that the oil extraction process increased the amount of β-sheet that is related to low availability of the protein (Table 5.4 and Table 5.3).

Theodoridou and Yu (2013) found α-helix:β-sheet ratio of 0.96 and Amide I:Amide II area ratio of 2.70 for canola meals. These results were close but lower than ours of 1.02 and 3.01, respectively (Table 5.3). Ban, Prates, Feng, Khan and Yu (2021) using Synchrontron FTIR reported a new line of brown canola seeds with α-helix:β-sheet ratio of 1.24 and Amide I:Amide II area ratio of 2.46. The canola seeds analyzed on our study showed lower α-helix:β-sheet ratio (1.07) and higher Amide I:Amide II area ratio (3.14). And Ban, Prates and Yu (2017) reported canola seeds having Amide I:Amide II height ratio of 1.73 (lower than ours 2.23) and α-helix:β-sheet ratio of 1.06. Although some variations are observed our results are in range with the literature.

The principal component analysis (PCA) of a few carbohydrate and protein related structures of canola seeds and meals are presented from Figure 5.1 to Figure 5.8 comparing Canada and China. The first principal component was able explain from 93 to 100% of the variability in the samples of the structures represented here and it was not possible to completely distinguish the samples from the two countries. Therefore, neither the seeds nor the meals are not completely different regarding the carbohydrate or protein spectral features between Canada and China.

Table 5.3. Using FTIR-ATR molecular spectroscopic technique to determine protein related molecular spectral features of canola meals and pellets: comparisons between companies, countries, and periods.

Items	Height		Ratio	Height		Ratio	Area			Ratio
	Amide I	Amide II	Amide I: Amide II	α-helix	β-sheet	α-helix: β-sheet	Amide	Amide I	Amide II	Amide I: Amide II
Canadian processing plants										
Plant 1 (M)	0.35	0.18	1.99	0.32	0.32	0.99	50.76	27.02	9.04	3.08
Plant 2 (M)	0.38	0.21	1.88	0.35	0.34	1.00	53.36	28.46	10.25	2.93
Plant 3 (P)	0.39	0.22	1.93	0.37	0.36	1.00	55.50	29.42	10.45	3.01
Plant 4 (P)	0.40	0.22	1.93	0.38	0.37	1.02	57.19	30.56	10.90	2.93
Plant 5 (P)	0.39	0.23	1.78	0.37	0.35	1.03	54.64	29.24	10.94	2.79
SEM	0.025	0.030	0.140	0.031	0.027	0.022	2.320	2.797	1.769	0.205
P value	0.193	0.283	0.481	0.124	0.132	0.538	0.236	0.209	0.422	0.664
Meals vs. Pellets										
SEM	0.082	0.075	0.405	0.091	0.072	0.112	11.14	5.830	4.237	0.729
P value	0.052	0.084	0.451	0.028	0.032	0.226	0.066	0.062	0.140	0.453
Chinese processing plants										
Plant A (M)	0.40^{a}	0.22^{a}	1.85^{b}	0.37^{ab}	0.37^{a}	1.01^{b}	54.08^{ab}	29.79^{ab}	10.86^{a}	2.85^{b}
Plant B (M)	0.41^{a}	0.22^{a}	1.92^{ab}	0.38^{a}	0.37^{a}	1.02^{b}	56.74^{a}	31.19^{a}	10.98^{a}	2.99^{ab}
Plant C (M)	0.41^{a}	0.21^{a}	2.02^{a}	0.39^{a}	0.37^{a}	1.05^{ab}	55.84^{a}	30.97^{a}	10.96^{a}	2.98^{ab}
Plant D (M)	0.41^{a}	0.21^{a}	1.99^{a}	0.39^{a}	0.36^{a}	1.08^{a}	56.48^{a}	30.93^{a}	10.71^{a}	3.03^{a}
Plant E (M)	0.37^{b}	0.20^{b}	1.91^{ab}	0.35^{b}	0.34^{b}	1.05^{ab}	51.86^{b}	28.40^{b}	10.06^{b}	2.99^{a}
SEM	0.018	0.026	0.152	0.021	0.025	0.019	1.605	2.620	1.758	0.221
P value	0.001	<0.001	0.013	<0.001	<0.001	0.014	0.001	0.001	0.004	0.017
Overall										
CA Plants	0.37	0.20	1.94	0.33	0.33	0.99	51.97	27.71	9.59	3.01
CH Plants	0.40	0.21	1.97	0.37	0.36	1.04	55.16	29.59	10.31	3.01
SEM	0.017	0.023	0.117	0.021	0.022	0.015	1.629	2.346	1.473	0.171
P value	0.011	0.175	0.585	0.001	0.012	0.008	0.038	0.019	0.149	0.977

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; M: Meals; P: Meals Pelleted; Overall: compares only meals; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; AIH: Amide I height; AIIH: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.

Table 5.4. Using FTIR-ATR molecular spectroscopic technique to determine protein related molecular spectral features of canola seeds: comparisons between companies, countries, and periods.

	Height		Ratio	Height		Ratio		Area		Ratio
Items	Amide I	Amide II	Amide I: Amide II	α-helix	β-sheet	α-helix: β-sheet	Amide	Amide I	Amide II	Amide I: Amide II
					Canadian processing plants					
Plant 1	0.33	0.16	2.19	0.33	0.30	1.10^{ab}	42.50	23.32	8.31	3.04
Plant 2	0.32	0.16	2.02	0.31	0.28	1.12^{a}	41.66	22.74	8.92	2.72
Plant 3	0.31	0.15	2.16	0.28	0.27	1.05^{bc}	41.11	21.72	7.80	3.10
Plant 4	0.33	0.15	2.36	0.31	0.31	1.01^{c}	42.84	23.86	7.91	3.37
Plant 5	0.34	0.15	2.37	0.32	0.30	1.06^{abc}	46.81	24.58	8.68	3.18
SEM	0.014	0.014	0.161	0.017	0.015	0.016	2.266	1.550	1.339	0.356
P value	0.533	0.471	0.067	0.234	0.229	<0.001	0.294	0.365	0.385	0.255
					Chinese processing plants					
Plant A	0.29	0.14	2.22	0.27	0.26	1.05	39.62	21.39	7.79	3.13
Plant B	0.31	0.14	2.42	0.29	0.28	1.06	43.21	22.98	7.98	3.42
Plant C	0.33	0.16	2.24	0.32	0.30	1.08	45.52	24.88	8.85	3.20
Plant D	0.33	0.16	2.14	0.31	0.29	1.08	43.28	23.82	9.18	2.81
Plant E	0.35	0.16	2.35	0.33	0.31	1.07	48.08	25.91	9.04	3.17
SEM	0.017	0.017	0.212	0.021	0.018	0.024	3.392	1.983	1.598	0.456
P value	0.135	0.100	0.414	0.136	0.083	0.736	0.248	0.205	0.152	0.607
					Overall					
CA Plants	0.33	0.15	2.23	0.31	0.29	1.07	42.68	23.23	8.22	3.14
CH Plants	0.33	0.15	2.28	0.31	0.29	1.07	44.79	23.87	8.38	3.17
SEM	0.009	0.013	0.151	0.014	0.014	0.010	1.752	1.358	1.311	0.317
P value	0.894	0.532	0.471	0.962	0.688	0.988	0.186	0.433	0.610	0.839

Means in the same column with different letters differ significantly ($P < 0.05$). SEM: standard error of the mean. CA: Canada; CH: China; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; AIH: Amide I height; AIIH: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.

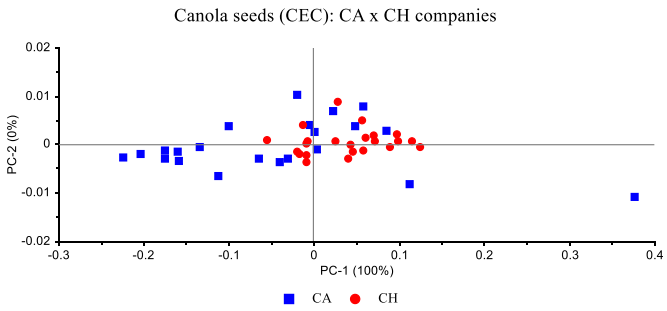

Figure 5.1. Principal Component Analysis (PCA) of CEC from canola seeds: comparison between Canada and China

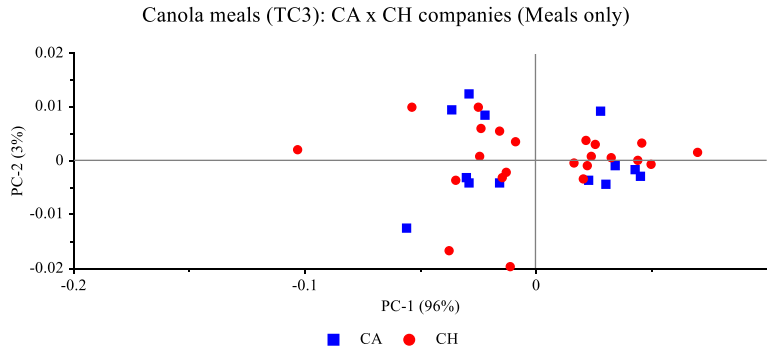

Figure 5.2. Principal Component Analysis (PCA) of TC3 from canola meals: comparison between Canada and China

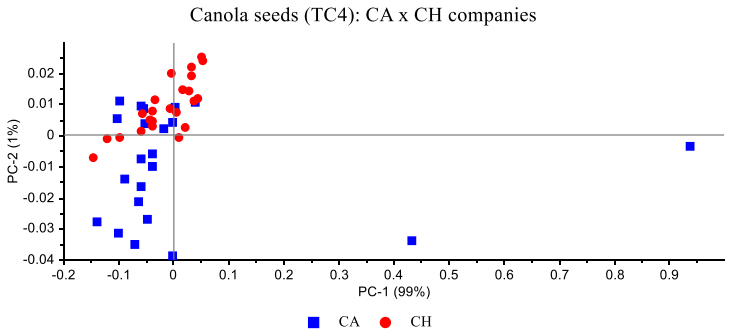

Figure 5.3. Principal Component Analysis (PCA) of TC4 from canola seeds: comparison between Canada and China

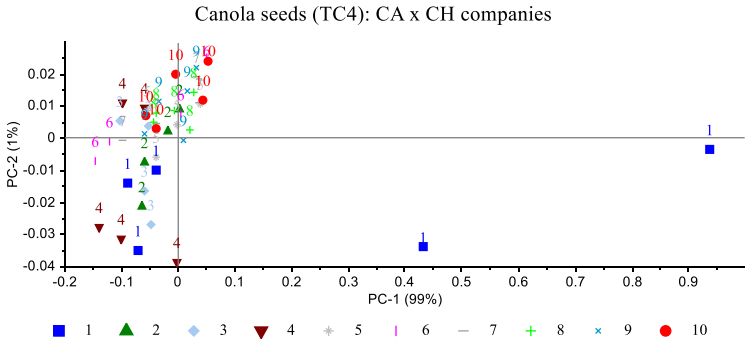

Figure 5.4. Principal Component Analysis (PCA) of TC4 from canola seeds: comparison between Canada and China by company

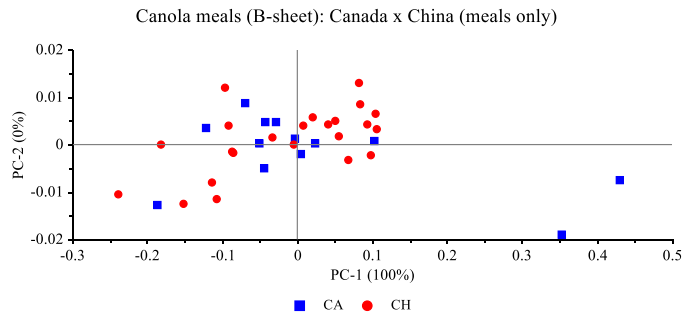

Figure 5.5. Principal Component Analysis (PCA) of β-sheet from canola meals: comparison between Canada and China

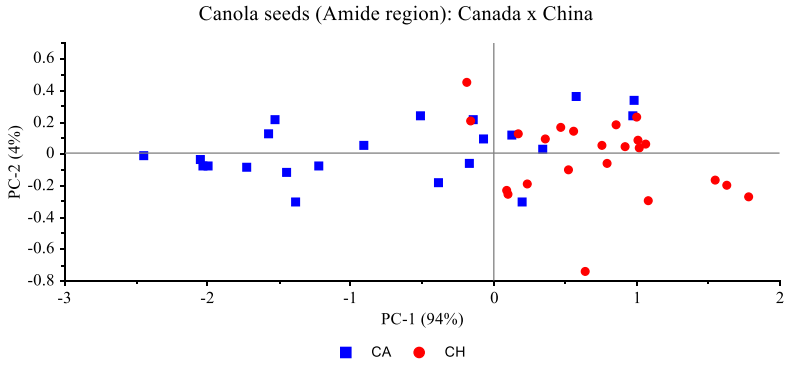

Figure 5.6. Principal Component Analysis (PCA) of Amide region from canola meals: comparison between Canada and China

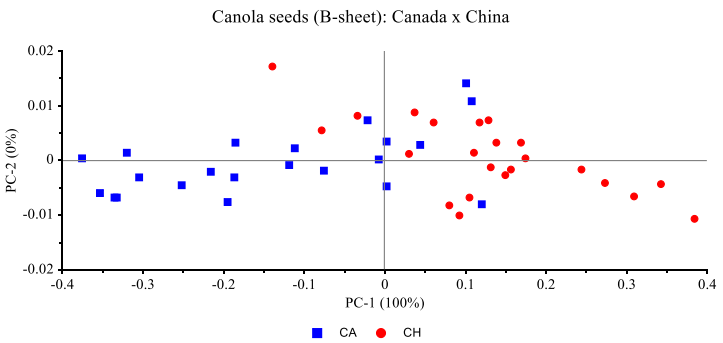

Figure 5.7. Principal Component Analysis (PCA) of β-sheet from canola seeds: comparison between Canada and China

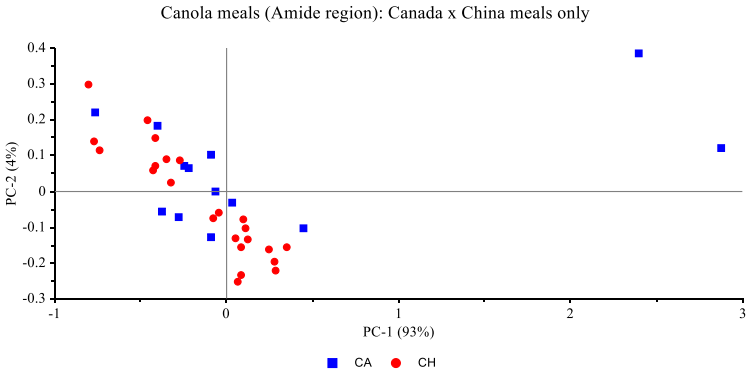

Figure 5.8. Principal Component Analysis (PCA) of Amide region from canola seeds: comparison between Canada and China

5.5. Chapter Summary

The FTIR-ATR analysis on the carbohydrate-related features showed a significant difference between Canadian companies and between the meals and pellets ($P<0.05$). Although not different within companies, when compared to Canada, the Chinese meals showed higher peak heights for total carbohydrate (TC3, TC4), cellulosic compounds (CEC), structural carbohydrates (STC2, STC3, and STC4), and areas for TC, CEC, and STC ($P<0.05$). Canadian canola seeds presented higher peaks for TC1, TC2, TC3, TC4, CEC, STC2, STC4, and TC area ($P<0.05$), while the ones from China showed a higher peak for SCT1 ($P=0.033$).

The analysis of the protein-related structures of canola seeds showed no differences between countries, and only the α-helix:β-sheet ratio was different among the Canadian companies ($P<0.001$). The Chinese meals, however, were all different between each other ($P<0.05$). Amide I height; α-helix and β-sheet heights and their ratio; and amide and amide I areas; were all higher in Chinese meals than Canadian meals and pellets ($P<0.05$). The Principal Component Analysis (PCA) reported showed the comparisons of some protein and some carbohydrate-related aspects of canola meals and seeds and it was not possible to completely differentiate the protein or the carbohydrate structures between countries. In conclusion, these results indicate that the seeds and meals processed in China are not different from the ones in Canada.

6. RELATIONSHIP BETWEEN THE MOLECULAR STRUCTURES SPECTRA FEATURES OF CANOLA SEEDS AND CANOLA MEALS AND NUTRIENT UTILIZATION AND AVAILABILITY TO DAIRY COWS

6.1. Abstract

Conventional methods for laboratory analysis are reliable but require excessive labor, time, chemicals, and amounts of samples. For these reasons, alternative methods have been explored. ATR/FTIR spectroscopy is technique that analyzes samples simply by shooting a bright light and measuring the absorbance at the mid-infrared range. Samples of canola seeds and meals were submitted to this technique and their protein and carbohydrate related spectral features were associated with the results obtained through the conventional methods of analyses for chemical and nutrient profiles, rumen degradable and intestinal digestible parameters. The procedure CORR was used at SAS 9.4 to determine the strength of the relationships between the carbohydrate-related molecular spectral profiles to the carbohydrate-relate characteristics of canola meals and seeds, as well as the protein-related molecular spectral profiles were related to the protein parameters of canola meals and seeds. Later the procedure REG at SAS 9.4 with best model variable selection was used to generate prediction equations based on the relationships observed on the samples. The STCA (ca. 1487.8 – 1190.8 cm^{-1}) was the carbohydrate structure that was most significant when related to carbohydrate parameters of canola meals ($P<0.05$, $r>0.50$). And TCA (ca. 1198.5 – 934.3 cm^{-1}) was the most significant when studying the carbohydrate parameters of canola seeds ($P<0.05$, $r>0.50$). Amide structures (ca. 1721.2 – 1480.1 cm^{-1}) were related to a few chemical and nutrient profiles, CNCPS, DVE/OEB, and NRC systems, and intestinal *in vitro* protein-related parameters in canola meals. Besides amide structures, α-helix height (ca. 1650.8 – 1643.1 cm^{-1}) and β-sheet height (ca. 1633.4 – 1625.7 $cm^{-1)}$, and the ratio

between them have shown to be related to many protein-related parameters in canola seeds. Multi-regression analysis resulted in moderate to high R^2 values for some protein related equations for canola seeds. Protein related equations for canola meals and carbohydrate related equations for canola meals and seeds resulted in weak R^2 and low P values ($P<0.05$). In conclusion, ATR/FTIR can be a useful resource to predict carbohydrate and protein-relates aspects of canola seeds and meals based on certain carbohydrate and protein spectral features inherent to canola seeds and meals.

6.2. Introduction

The dairy production system, especially in Canada, uses canola meal, rather than the seeds a source of protein because canola seeds are largely crushed for its oil content generating the meal as a co-product. The literature indicates that changes in temperature and time of harvesting can alter the chemical composition of canola seeds and different processing methods can alter the composition of canola meals (Newkirk, 2011). Furthermore, the chemical composition of feedstuffs is indispensable in animal nutrition for feeds account for 70-85% of the costs in animal production (Viljoen, 2003).

Wet laboratory analyses methods and *in vivo* studies require intensive labor, high amounts of samples and long hours. And each day the industry brings forth a new variety of plant, a different method of processing etc. and all can affect the final product that is consumed by the animals, therefore determining the chemical composition is required and the faster this information can be obtained, the faster the industry can improve, and better animal performance and increased profits can be observed. Therefore, a fast method of analysis for canola seeds and meals would be helpful in the dairy industry, saving time in analysis and money in manipulating diets that are taking into consideration the real specific characteristics of the ingredients being used.

As an alternative to time consuming wet laboratory analysis, different infrared spectroscopy methods have gained space in animal nutrition (Viljoen, 2003; Ban, Prates, and Yu, 2017; Chen, Zhang, and Yu, 2014; etc.). Spectroscopy is being used because it studies matters though its interaction with light quickly and without damaging the sample. The attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analyzes the interaction of matter with infrared light on the mid-infrared region (ca. 4000 to 800 cm^{-1}) in a quick and non-destructive

manner (Ban, Prates and Yu, 2017). This is different from the wet analyses that use chemicals and procedures that can damage structures and alter the composition and digestibility of feeds (Theodoridou and Yu, 2013).

ATR-FTIR can help us learn not only about the composition of an ingredient but also what kind of response that ingredient has when fed to a ruminant. Therefore, to understand how the intrinsic molecular structures of canola seeds and meals relate to the chemical composition, energy profile, degradability, and digestibility in the gastrointestinal tract of dairy cows is an advantage for the industry and was the objective of this study.

6.3. Materials and Methods

6.3.1. Sampling

Samples from canola bio-oil processing plants in Canada and China were collected by the Canola Council of Canada in 2016. Five different companies in Canada provided samples from seeds used and meals produces in five different batches. As well as five different companies in China provided samples from the seeds and meals from five different batches. Each company's quality control laboratory provided the samples that were later analyzed at the University of Saskatchewan in Canada.

Chemical analyses followed the AOAC Official Methods of Analysis (2019); the *in situ* study required the rumen incubation of 7g samples at 0, 2, 4, 8, 12, 24 and 48h in four Holstein cows following the animal care guidelines and approved by the ethics committee of the University of Saskatchewan; the *in vitro* study followed the three-step procedure by Calsamiglia and Stern (1995); and spectral analysis used the ATR-FTIR technique (attenuated total reflectance Fourier transform infrared spectroscopy) to study carbohydrate and protein-related molecular structures. All procedures and analyses were realized at the University of Saskatchewan and are reported in detail in the previous chapters.

6.3.2. Statistical analysis

To study the relationship between the various spectral features to the chemical and energy profiles, and rumen and intestinal availability and digestibility, the data of interest were analyzed using the procedure CORR on SAS® 9.4 (SAS Institute, USA).

The procedure REG on SAS® 9.4 (SAS Institute, USA) was used for the multi-regression analysis to create prediction equations based on the data collected during this study. Only the significant model equations are represented here (R^2>0.60.

6.4. Results and Discussion

6.4.1. Relationship Study on Carbohydrate-related Spectral Features and Chemical and Nutrient Profiles and Rumen degradation and Intestinal Digestion

In a correlation analysis, the P value lower than the α significance level (0.05) indicates that there is a linear relationship between the variables analyzed, and the r value will determine the strength of this relationship, where r=1 or -1, is a perfect relationship; r=0.8 or -0.8, indicate a strong relationship; r=0.6 or -0.6, indicate a moderate relationship; r=0, indicates absence of linear relationship (Frost, 2018). Represented from Table 6.1 to Table 6.9 are the correlation between carbohydrate-related molecular structures and variables from chemical and energy profiles, CNCPS, NRC, and DVE/OEB systems that showed significance (P<0.05).

The structural carbohydrates spectral peak area (STCA) is the molecular structure that seems to have linear relationships with many of the characteristics of canola meals studied. It is related to the contents of cellulose and lignin (Table 6.1), digestible fiber fractions (Table 6.3), effective degradability of protein (EDCP) and microbial protein synthesized in the rumen based on energy (MREE) (Table 6.5), total digestible neutral detergent fiber (tdNDF) and feed milk value (Table 6.6), and with endogenous crude protein (ECP) and ECP truly absorbed in the small

intestine (AECP) (Table 6.8). While other structures were also related, STCA was related to at least one aspect of each studied profile or system.

The total carbohydrate area (TCA) is the carbohydrate-related structure that was found to be related to many characteristics of canola seeds in this study. It is related to the sugar content (Table 6.2), rumen degradable and undegradable fractions of water soluble carbohydrates (RDCA4 and RUCA4) (Table 6.4), and to endogenous crude protein (ECP) and ECP truly absorbed in the small intestine (AECP) (Table 6.9). The cellulosic compounds area (CECA) was linearly related to neutral detergent aspects (NDF and tdNDF) (Table 6.2 and Table 6.7). None of the carbohydrate molecular structures studied on this project appeared to have a linear relationship with any of the DVE/OEB system variables for canola seeds ($P>0.05$, values not represented).

This study shows that it is possibl to relate many feed characteristics and ruminal and intestinal responses of canola seeds and meals fed to dairy cows from their carbohydrate-related spectral profiles revealed through molecular analysis using the ATR/FTIR technology.

Table 6.1. Correlation between FTIR carbohydrate structures and the carbohydrate portions of the chemical profile of canola meals.

	TC1H		TC2H		TC3H		CECH		STCA	
	r	P value	r	P value	r	P value	r	P value	r	P value
NDF (%DM)			-0.57	0.027	-0.52	0.049				
Hemicellulose (%DM)			-0.54	0.036						
Cellulose (%DM)							0.55	0.035	0.64	0.011
ADL (%NDF)									-0.60	0.018

NDF: neutral detergent fiber; ADF: acid detergent fiber; TCxH: total carbohydrate peak height; CECH: cellulosic compounds peak height; STCA: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; r: correlation coefficient using Spearman. Missing values had P>0.05.

Table 6.2. Correlation between FTIR carbohydrate structures and the carbohydrate portions of the Chemical Profile of canola seeds.

	TC2H		STC1H		CECA		TCA	
	r	P value	r	P value	r	P value	r	P value
NDF (%DM)	-0.56	0.031			-0.53	0.044		
ADL (%DM)			0.66	0.008				
NFC (%CHO)								
Sugar (%DM)	0.56	0.030	-0.58	0.022			0.69	0.004

NDF: neutral detergent fiber; ADF: acid detergent fiber; TCxH: total carbohydrate peak height; CECH: cellulosic compounds peak height; STCA: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; r: correlation coefficient using Spearman. Missing values had P>0.05.

Table 6.3. Correlation between FTIR carbohydrate structures and the carbohydrate portions of the CNCPS system of canola meals.

	TC2H		CECH		STC1H		STC2H		CECA		TCA		STCA	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
CB3 (%CHO)	-0.56	0.029			0.60	0.018			0.55	0.032				
RDCB3	-0.52	0.045			0.54	0.037			0.53	0.041			0.56	0.028
Total RDC			0.55	0.033			0.68	0.005						
RUCA4														
RUCB3	-0.52	0.045			0.54	0.037			0.53	0.041			0.56	0.028
RUCC											-0.52	0.046		

RDCB3: ruminally degradable digestible fiber; TotalRDC: total ruminally degradable carbohydrates; RUCA4: ruminally undegradable water soluble carbohydrates; RUCB3: ruminally undegradable digestible fiber; RUCC: indigestible fiber; TCxH: total carbohydrate peak height; CECH: cellulosic compounds peak height; STCA: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; r: correlation coefficient using Spearman. Missing values had P>0.05.

Table 6.4. Correlation between FTIR carbohydrate structures and the carbohydrate portions of the CNCPS system of Canola seeds.

	TC2H		TC3H		STC1H		TCA	
	r	P value	r	P value	r	P value	r	P value
CB2 (%CHO)					0.56	0.030		
CC (%CHO)					0.65	0.008		
RDCA4	0.56	0.030			-0.58	0.022	0.69	0.004
RUCA4	0.56	0.029	0.52	0.048	-0.57	0.027	0.69	0.004

CB2: soluble fiber; CC: unavailable fiber; RUCA4: ruminally undegradable water soluble carbohydrates; TC: total carbohydrate; STC: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; H: peak height; A: peak area; r: correlation coefficient using Spearman. Missing values had P>0.05.

Table 6.5. Correlation between FTIR carbohydrate structures and the DVE/OEB System for canola meals.

	STCA	
	r	P value
EDCP	0.62	0.014
MREE	0.62	0.014

EDCP: Effective degradability of CP; MREE: microbial protein synthesized in the rumen based on the energy available; STC: structural carbohydrate; A: area; r: correlation coefficient using Spearman.

Table 6.6. Correlation between FTIR carbohydrate structures and the Energy Profile of canola meals.

	TC3H		STCA	
	r	P value	r	P value
tdNDF			0.65	0.008
Estimated Milk	0.55	0.034		

tdNDF: total digestible neutral detergent fiber; Estimated milk: estimated milk production based on energy TC: total carbohydrate; STC: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; H: peak height; A: peak area; r: correlation coefficient using Spearman. Missing values had P>0.05.

Table 6.7. Correlation between FTIR carbohydrate structures and the Energy Profile of canola seeds.

	CECA	
	r	P value
tdNDF	-0.54	0.038

tdNDF: total digestible neutral detergent fiber; CEC: cellulosic compound area; r: correlation coefficient using Spearman.

Table 6.8. Correlation between FTIR carbohydrate structures and the NRC System for canola meals.

	STC2H		STC3H		STC4H		STCA	
	r	P value	r	P value	r	P value	r	P value
AECP	-0.58	0.024	-0.80	<0.001	-0.75	0.001	-0.86	<0.001
ECP	-0.58	0.025	-0.80	<0.001	-0.75	0.001	-0.85	<0.001

AECP: truly absorbed ECP in the small intestine; ECP: Endogenous crude protein in the small intestine; STC: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; H: peak height; A: peak area; r: correlation coefficient using Spearman.

Table 6.9. Correlation between FTIR carbohydrate structures and the NRC System for canola seeds.

	TC2H		TC3H		TC4H		CECH		STC1H		STC4H		TCA	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
AECP	0.57	0.027	0.73	0.002	0.56	0.029	0.72	0.002	-0.96	<0.001	0.55	0.032	0.79	<0.001
ECP	0.60	0.019	0.74	0.002	0.57	0.026	0.75	0.001	-0.96	<0.001	0.58	0.024	0.81	<0.001

AECP: truly absorbed ECP in the small intestine; ECP: Endogenous crude protein in the small intestine; TC: total carbohydrate; CEC: cellulosic compounds; STC: structural carbohydrate area; 1, 2, 3 and 4: correspond to the different peaks; H: peak height; A: peak area; r: correlation coefficient using Spearman.

6.4.2. Relationship Study on Protein-related Spectral Features and Chemical and Nutrient Profiles and Rumen degradation and Intestinal Digestion of canola seeds and meals

The linear relationship study between protein structures revealed through the ATR/FTIR technique and chemical characteristics of canola meals and seeds that were significant are presented from Table 6.10 to Table 6.19. Many variables from canola meals and seeds showed to be related with amides areas and heights in the present study. Weak correlations are not represented on these tables.

Amides peak area and amides height are related to the soluble crude protein content of both canola meals and seeds (Table 6.10 and Table 6.11), but stronger relationships were observed on canola seeds ($r=0.64$, $P<0.001$, for peak area; $r=0.62$, $P<0.001$, for amide height) (Table 6.11). Slowly degradable protein fractions (PB2 and RDPB2) of canola meals are negatively related to Amide area, and moderately degradable protein (PB1) is also negatively related to amide area ratio and amide height in canola meals (Table 6.12). Only the truly digested protein in the small intestine (DVE) ($r=0.57$, $P=0.026$) and estimated milk production (DVE FMV) ($R=0.56$, $P=0.028$) of canola meals seemed to be related to the height of amide II (Table 6.14).

Strong relationships can be observed between many protein structures and soluble protein fractions (PA2, RDPA2, and RUPA2), moderately degradable fractions (PB1, RDPB1, and RUPB1), unavailable protein (PC), total protein (TP), and total degradable (Total RDP) and undegradable protein (Total RUP) (Table 6.13) of canola seeds. Residue at 0h and the soluble fraction of canola seeds are related to peak area, amide II area, amide areas ratio, amide I and II heights, and β-sheet height (Table 6.15).

Different fractions of the in vitro digestibility showed relationships in canola seeds and meals. The intestinal digestibility of proteins (IDP) of canola meals was related to the height of Amide II ($r=0.63$, and $P=0.012$) (Table 6.16). While both the digestibility of bypass dry matter (dBDM) ($r=0.67$, $P=0.007$) and the intestinally absorbable feed protein (IADP) ($r=0.65$, $P=0.009$) were related to the α-helix:β-sheet ratio (Table 6.17). Amide II height was related to microbial protein (MP) on canola meals ($r=0.53$, $P=0.043$) (Table 6.18) and to endogenous crude protein (ECP) ($r=0.61$, $P=0.016$) and ECP truly absorbed in the small intestine (AECP) ($r= 0.60$, $P=0.019$)

on canola seeds (Table 6.19). On canola seeds, AECP was also related to α-helix (r= 0.63, P=0.013) and to α-helix:β-sheet ratio (r= 0.88, P<0.001), similarly ECP was also related to α-helix (r=0.63, P=0.012) and to α-helix:β-sheet ratio (r=0.84, P<0.001) (Table 6.19).

Theodoridou and Yu (2013) studied the correlation of canola meals and presscake to protein structures and they also found that amide I and II areas and their ratio were related to NDIP (r=0.95, P=0.051), PB1 (r=-0.76, P=0.244), PB2 (r=0.82, P=0.188), IDP (r=0.89, P=0.107), MP (r=0.99, P=0.006). The high r values that they obtained show a tendency for a strong relationship, but the high P values indicate that a higher sample size is necessary to confirm those relationships (Brendan Oconnor, 2011). Similar to our results, Huang (2015) found relationships between the amide I and II areas, heights and their ratios and CP, SCP, PA2, PB1, tdCP, S, D, and TDP in pelleted canola meals (r>0.76 and P<0.05), but did not find correlation between α-helix height, β-sheet height, and α-helix:β-sheet ratio and any protein parameters of canola meals.

These results, along with ours, indicate that the various processes for oil extraction, desolventizing of the meals, and pelleting may affect the protein structures of the meals differently, even if the companies use similar processes. Or simply, they indicate that repeating the study with higher sample sizes would improve the results and give us a clearer understanding of the correlations between protein structures and the characteristics of canola meals. Although based on our results it seems to be easier to relate protein spectral structures with the protein structures of canola seeds, because more frequent and stronger relationships could be observed for the seeds than for the meals, more repetition could only help to support the results presented here.

Table 6.10. Correlation between FTIR protein structures and the protein portions of the chemical profile of canola meals.

	SCP (%CP)		NDIP (%CP)		NDIP (%DM)	
	r	P value	r	P value	r	P value
Peak area	0.51	<0.001	-0.52	<0.001	-0.54	<0.001
Height	0.53	<0.001				

DM: dry matter; CP: crude protein; SCP: soluble crude protein; NDIP: neutral detergent-insoluble crude protein; Peak area: Amide I and II peak area; Height: ratios of amide I and II heights; r: correlation coefficient using Spearman.

Table 6.11. Correlation study between FTIR protein structures and the protein portions of the Chemical Profile of Canola seeds.

	SCP (%CP)	
	r	P value
Peak area	0.64	<0.001
Area ratio	-0.52	<0.001
Height	0.62	<0.001

DM: dry matter; CP: crude protein; SCP: soluble crude protein; Peak area: Amide I and II peak area; Area ratio: ratios of amide I and amide II areas; Height: ratios of amide I and II heights; r: correlation coefficient using Spearman.

Table 6.12. Correlation study between FTIR protein structures and the protein portions of the CNCPS System Profile of Canola meals.

	PB2 (%CP)		PB1 (%CP)		PB1 (%TP)		PB2 (%TP)		RDPB2 (%DM)	
	r	P value	r	P value	r	P value	r	P value	r	P value
AII	-0.52	0.046					-0.55	0.034	-0.52	0.049
Area ratio			-0.57	0.025	-0.57	0.028				
Height					-0.53	0.041				

DM: dry matter; CP: crude protein; PB1: moderately degradable protein; PB2: slowly degradable protein; RD: Rumen degradable; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; Height: ratios of amide I and II heights; r: correlation coefficient using Spearman.

Table 6.13. Correlation study between FTIR protein structures and the protein portions of the CNCPS Profile of Canola seeds.

	PA2		PC		PB1		TP		RDPA2		RDPB1	
	r	P value	r	P value	R	P value	r	P value	r	P value	r	P value
Peak area	0.74	0.002			-0.63	0.012			0.75	0.001	-0.64	0.010
AI	0.76	0.001			-0.64	0.010			0.76	0.001	-0.65	0.009
AII	0.56	0.030							0.59	0.021		
AIH	0.65	0.008			-0.56	0.030			0.67	0.006	-0.57	0.026
AIIH	0.57	0.028							0.60	0.019	-0.52	0.047
Height			0.58	0.023			-0.58	0.023				
Alpha	0.74	0.002			-0.69	0.004			0.76	0.001	-0.68	0.005
Beta	0.55	0.034							0.55	0.032		
Ratio												

	TOTAL RDP		RUPA2		RUPB1		RUPC		Total RUP	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.76	0.001	0.75	0.001	-0.64	0.001			-0.74	0.002
AI	0.75	0.001	0.76	0.001	-0.65	0.001			-0.78	<0.001
AII	0.60	0.017	0.59	0.021					-0.59	0.022
AIH	0.69	0.004	0.67	0.006	-0.57	0.026			-0.65	0.009
AIIH	0.64	0.010	0.60	0.019	-0.52	0.047	-0.53	0.041	-0.59	0.021
Height							0.56	0.031		
Alpha	0.79	<0.001	0.76	0.001	-0.68	0.005			-0.69	0.005
Beta	0.56	0.030	0.55	0.032					-0.56	0.029
Ratio	0.57	0.027								

DM: dry matter; CP: crude protein; PA2: soluble true protein; PB1: moderately degradable protein; PB2: slowly degradable protein; PC: unavailable crude protein; Total RDP: total rumen degradable protein; RD: rumen degraded; RU: rumen undegraded; Total RUP: total rumen undegradable protein; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; AIH: Amide I height; AIIH: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet; r: correlation coefficient using Spearman.

Table 6.14. Correlation study between FTIR protein structures of Canola meals and the DVE/OEB system.

	DVE		DVE FMV	
	r	P value	r	P value
AIIH	0.57	0.026	0.56	0.028

DVE: truly digested protein in the small intestine; DVE FMV: estimated milk production based on the DVE system in kg milk/kg DM feed. AIIH: Amide II height; r: correlation coefficient using Spearman.

Table 6.15. Correlation study between FTIR protein structures and the protein portions of the in situ rumen incubation of canola seeds.

	Kd (%/h)		Fr (%)		Residue 0h (%)		S (%)		U (%)		BCP (%)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area					-0.52	0.046	0.52	0.046				
AII					-0.65	0.008	0.65	0.008				
Area ratio	0.60	0.019			0.66	0.007	-0.66	0.007	0.61	0.017		
AIH					-0.60	0.018	0.60	0.018				
AIIH					-0.58	0.023	0.58	0.023				
Beta					-0.54	0.039	0.54	0.039				
Ratio	-0.76	0.001	-0.60	0.019					-0.65	0.009	0.52	0.046

Fr: Fermentation rate; Residue 0h: CP residue at 0h of rumen incubation; Kd: the degradation rate of D fraction; S: soluble fraction; U: rumen undegradable fraction; BCP: Bypass CP; Peak area: Amide I and II peak area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; AIH: Amide I height; AIIH: Amide II height; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

Table 6.16. Correlation study between FTIR protein structures and the protein portions of the in vitro of canola meals.

	IDP (%RUP)	
	r	P value
AIIH	0.63	0.012

AIIH: amide II height; IDP: intestinal digestibility of protein; r: correlation coefficient using Spearman.

Table 6.17. Correlation study between FTIR protein structures and the protein portions of the in vitro of canola seeds.

	dBDM (%)		IADP (g/Kg DM)	
	r	P value	r	P value
Ratio	0.67	0.007	0.65	0.009

dBDM: Digestibility of bypass DM; IADP: Intestinally absorbable feed protein; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

Table 6.18. Correlation study between FTIR protein structures of canola meals and the NRC model.

	MP	
	r	P value
AIIH	0.53	0.043

MP: metabolizable protein; AIIH: Amide II height; r: correlation coefficient using Spearman.

Table 6.19. Correlation study between FTIR protein structures of Canola seeds and the NRC model.

	AECP		ECP	
	r	P value	r	P value
AII	0.54	0.040	0.55	0.033
AIIH	0.60	0.019	0.61	0.016
Alpha	0.63	0.013	0.63	0.012
Ratio	0.88	<0.001	0.84	<0.001

AECP: truly absorbed ECP in the small intestine; ECP: endogenous protein in the small intestine; Peak area: Amide I and II peak area; AII: Amide II area; AIH: Amide I height; AIIH: Amide II height; Alpha: α-helix height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

6.4.3. *Prediction of Nutrient Supply and Rumen and Intestinal Digestion of Canola Seeds and Meals Using Unique Molecular Spectral Features*

Table 6.20. Best model variables selection in multi-regression analysis to predict Canola protein parameters from FTIR protein structures.

Variables (Y)	Prediction equation model: Y=a + b1 x X1 + b2 x X2 …	R^2	RSD	*p* value
	Canola seeds			
PA2 (%CP)	Y = 33.36 + 5.24 x AI – 367.58 x Ratio	0.72	3.36	<0.001
RDPA2 (%DM)	Y = 5.88 + 0.83 x AI – 59.31 x Alpha	0.68	0.56	0.001
RDPB1 (%DM)	Y = 4.44 – 0.47 x AI + 34.84 x Beta	0.64	0.33	0.002
RUPA2 (%DM)	Y = 2.35 + 0.33 x AI -23.72 x Beta	0.68	0.23	0.001
RUPB1(%DM)	Y = 6.70 + 0.70 x AI + 52.15 x Beta	0.65	0.50	0.002
Total RUP (%DM)	Y = 12.59 – 0.40 x AI + 25.35 x Beta	0.75	0.25	<0.001
AECP (%DM)	Y = 3.96 – 0.15 x Area + 0.76 x Ratio	0.82	0.03	<0.001
ECP (%DM)	Y = 9.97 – 0.41 x Area + 1.90 x Ratio	0.81	0.07	<0.001
	Canola meals			
CP (%DM)	Y = 38.11 – 15.61 x A2H + 20.90 x Alpha	0.23	0.89	0.004
TD (%CP)	Y = 37.09 – 17.80 x A2H + 22.43 x Alpha	0.24	0.90	0.004
TDN_{1x}	Y = 62.78 + 13.64 x A2H	0.23	1.38	<0.001
DE_{1x}	Y = 3.09 + 0.66 x Alpha	0.27	0.06	<0.001
DEp_{3x}	Y = 3.03 + 0.41 x Alpha	0.27	0.04	<0.001
ME_{3x}	Y = 2.54 + 0.54 x Alpha	0.27	0.05	<0.001
MEp_{3x}	Y = 2.61 + 0.41 x Alpha	0.27	0.04	<0.001
Nem_{3x}	Y = 1.66 + 0.45 x Alpha	0.25	0.04	<0.001
NEg_{3x}	Y = 1.03 +0.40 x Alpha	0.26	0.04	<0.001
NEL_{p3x}	Y = 1.65 + 0.29 x Alpha	0.25	0.03	<0.001
Estimated Milk Value (FMV)	Y = 2.46 + 0.44 x Alpha	0.26	0.04	<0.001

RSD: residual standard deviation; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet; AIIH: Amide II height; AI: Amide I area; Height: AIH:AIIH ratio; Area: AI:AII areas ratio; CP: crude protein; TDCP: Total digestible CP; PA2: soluble true protein; PB1: moderately degradable protein; Total RDP: total rumen degradable protein; Total RUP: total rumen undegradable protein; TDN: total digestible nutrients; DE: digestible energy; ME: metabolizable energy; NEm: net energy for maintenance,NEg_{3x}: net energy for gain; NEL: net energy for lactation.

Table 6.21. Regression analysis to predict Canola carbohydrate parameters from FTIR carbohydrate structures.

Variables (Y)	Prediction equation model: Y=a + b1 x X1 + b2 x X2 …	R^2	RSD	*p* value
	Canola meals			
CEL (%DM)	Y = 6.24 + 0.28 x STCA	0.37	0.66	0.017
Total RDC	Y = 19.55 + 90.09 x STC2H	0.35	1.01	0.021
MREE	Y = 52.46 + 1.61 x STCA	0.34	4.04	0.022
	Canola seeds			
HEMI (%DM)	Y = 15.72 – 0.58 x STCA	0.30	1.30	0.034
DVE	Y = 37.28 + 129.46 x TC4H	0.31	6.55	0.031
DVE FMV	Y = 0.76 + 2.62 x TC4H	0.31	0.13	0.030

RSD: residual standard deviation; CEL: cellulose; HEMI: hemicellulose; Total RDC: total rumen degradable carbohydrates; DVE: truly digested protein in the small intestine; DVE FMV: estimated milk production based on the DVE system in kg milk/kg DM feed; MREE: microbial protein synthesized in the rumen based on the energy available; STC: structural carbohydrate; TC: total carbohydrate; H: height; A: area; Numbers 2 and 4 correspond to different peaks.

Multiple regression analysis is used to verify the strength of the relationship between a dependent variable and several predictor variables, quantifying and statistically eliminating the effect of other predictors (Petchko, 2018). In our study, carbohydrate and protein-related spectral structures were used to predict chemical, degradable, and digestible characteristics of canola meals and seeds. However, the most significant prediction equations were relating protein-related structures to ruminal degradability and intestinal digestibility aspects of canola seeds (Table 6.20). The area of Amide I along with either the heights of α-helix or β-sheet or their ratio seem to be good predictors of rumen degradable and undegradable soluble and moderately soluble protein fractions (PA2, RDPA2, RDPB1, RUPA2, and RUPB1), and of the total rumen undegraded protein (Total RUP) in canola seeds ($P<0.05$, $R^2 \geq 0.65$). These results are important because in ruminants there is an extensive use of nitrogen compounds by the ruminal microbiota and that affects the quantity and quality of protein available for digestion in the small intestine. Being able to predict with more confidence how much of the protein in the canola seeds will be available for the animal to use, is extremely helpful in animal nutrition.

Although a low R^2 means variation in the results, this variability shows a trend behavior of the variables studied. All the prediction equations for canola meals using protein-related structures showed low R^2 but they also showed very low P values (Table 6.20). This indicates that even not being too precise, a trend is observed between those variables, and α-helix height is the protein-related structure that appears to be a good predictor for many energy-related variables. Crude protein and total digestible crude protein also showed a trend to be predicted by the α-helix height and amide II height. A similar response can be observed between some carbohydrate-related structures and some aspects of canola meals and seeds (Table 6.21). These results clearly show a pattern and further analysis with more data would likely increase the R^2 values and give more assurance to the users of these equations.

6.5. Chapter Summary

The correlation study between carbohydrate spectral features and canola meals and seeds showed that STCA commonly appear to be related to canola meals and TCA to canola seeds features. And the correlation between protein spectral features and canola meals and seeds aspects showed strong relationships with the amide region of both seeds and meals, but more and stronger relationships were observed on canola seeds. These results indicate that the carbohydrate and protein structures obtained with FTIR-ATR have been proven to be related to aspects of canola seeds and meals' chemical and nutrient profiles, as well as rumen degradable and intestinal digestibility characteristics. Also, the multi-regression analysis of canola meals and seeds and carbohydrate and protein-related molecular structures showed trends between protein-related structures for the canola meals equations ($P \leq 0.004$ and $R^2 \geq 0.23$). However, high R^2 (>0.64) and low P values (≤0.002) observed for canola seeds using protein molecular structures indicate that a higher trust can be put onto those equations.

7. GENERAL DISCUSSION AND SUMMARY

Canola and its products (oil and meal) are of high importance for the Canadian economy. And canola has been studied and largely explored specially in Western Canada to be used and consumed in Canada and exported to many countries. China is the biggest consumer of canola seeds produced in Canada and a significant consumer of canola meal and oil. China is also a large producer of a rapeseed, like canola, but not with the same lower levels of erucic acid (that reduces the palatability of the oil for human consumption) and glucosinolates (that reduces the palatability for animals' consumption of the meal). For this study, we analyzed samples of canola seeds and meals that were collected from five crushing companies in Canada and another five companies in China with five replicates from different batches. Samples were made available to the Canadian Canola Council by each company's quality control laboratory. Our project aimed at characterizing canola seeds and meals from different companies in Canada and in China.

We observed variations in the chemical profiles of canola seeds and meals. Canadian plants showed higher DM ($P=0.008$), NDICP ($P<0.001$), cellulose ($P<0.001$), and NDF ($P=0.004$) and Chinese plants higher SCP ($P=0.003$). As expected, due to processing of the seeds in different plants, meals presented more variations than the seeds. DM ($P<0.001$), ash ($P<0.001$), NDICP ($P<0.001$), and ADL ($P<0.001$) were higher in Canadian plants, and CP ($P=0.003$), SCP ($P<0.001$) and cellulose ($P<0.001$) were higher in Chinese plants. In the same chapter, we saw that the energy profile of canola meals in China presented higher tdNDF and tdCP ($P<0.001$, each), and Canada presented higher tdNFC ($P=0.006$). And canola seeds in Canada had higher tdNDF ($P=0.023$). The application of the CNCPS 6.5 system showed that Chinese crushers had higher PA2 ($P<0.001$) and lower PB2 ($P<0.001$) than Canadian crushers in canola meals. However, canola seeds in Chinese companies had higher amounts of PB2($P<0.001$). CA4 ($P=0.040$) was higher in meals in China, and CB2 ($P=0.010$) and CC ($P<0.001$) were higher in meals in Canada. CA4 was also higher in CH seeds ($P=0.022$). Other similar differences could also be observed on the ruminal degradable and undegradable fractions of canola meals and seeds. It is important to also consider a few points: meals that are mash are distributed locally, while the pelleted are sent longer distances and the pelleting process include the addition of screenings, gums, and high temperatures to form the pellets. Each of these can affect the characteristics of the meal. The addition of gums to the pellets

can increase the amount of EE in the meals, while the addition of screening might alter the protein composition, and the use of high temperatures in the pelleting process can increase the amount of RUP due to the possible Maillard reactions caused by the interaction of sugar and amino acids. Three out of the five crushing companies in Canada provided samples that were pelleted and all five crushing companies in China provided mash meals.

Besides the chemical composition, the ruminal degradability and intestinal digestibility are extremely important when studying feeds because the difference in animal performance is based on what is available to the animal. The *in situ* degradation of DM and CP parameters of both canola seeds and meals were similar in the comparison between the two countries. The CP rumen degradation of meals from Canadian plants, only at 24 (P=0.042) and 48h (P=0.040) were lower than the Chinese plants, while performing similarly on the other time-points, and for all time-points for the seeds. Oppositely, the DM rumen degradation of meals was only different at 0h (P=0.016) but both countries performed similarly in all other time-points and for the seeds. Deacon et al. (1988) found similar disappearance of DM in rumen between 0 and 8h of incubation of canola seeds, but for 12 and 24h they reported higher disappearance than us. And when comparing the disappearance of CP of the canola seeds, they reported values much higher than ours. These differences can be explained by them not using any procedures for correct the statistical analysis, judging these data are non-linear we, for instance, used procedure NLIN at SAS to account for that.

From the *in vitro* intestinal digestibility study, only the TDDM (P=0.018) was higher in China and IDP (P=0.016) was higher in canola meals from Canada, and all aspects were similar for the seeds. McKinnon, Olubobokun, Mustafa, Cohen, and Christensen (1995) found similar TDDM of canola meals in their study, but higher TDP than us. However, on their study they considered the fecal residue while we just used the *in vitro* procedure. The dutch DVE/OEB system revealed that MREE (P=0.011) and DVME (P=0.011) were higher in Chinese meals, while seeds presented the same performance. According to this system, canola meals from crushing plants in China had more energy available to the rumen microbes, thus there was higher microbe synthesis in the rumen and consequently a higher digestibility and absorbance of these in the intestine, which guarantees a regular supply of amino acids to the animals. The NRC model revealed a higher supply of endogenous protein from canola meals from Canadian crushing companies and consequently,

higher digestion and absorption of these in the small intestine. As the DVE/OEB system, seeds obtained from crushers from either country performed similarly in the NRC model. And the feed milk value based on the DVE/OEB, NRC, and energy systems of calculations showed they were not different between countries for neither meals nor seeds, meaning that feeding dairy cows with canola seeds or meals crushed either in Canada or in China would result in similar milk production.

Wet chemical analyses take time, require the use of harsh chemicals, long hours and still fail to answer what the actual availability will be to the animals. Molecular spectroscopy comes handy in this case because it requires no preparation of the samples and shows the results in seconds and with these, we can assess the molecular structures that are responsible for the availability or not to digestion. For instance, the presence of high amounts of β-sheet indicate a reduced availability of proteins to gastrointestinal enzymes to act on. Our study showed that different peaks and areas of various molecular structures were related to either protein or carbohydrate in the samples analyzed. Meals from Chinese crushers showed higher peak heights for TC3, TC4, CEC, STC2, STC3, and STC4, and areas for TC, CEC, and STC ($P<0.05$). Canola seeds from Canadian crushers presented higher peaks for TC1, TC2, TC3, TC4, CEC, STC2, STC4, and TC area ($P<0.05$), while Chinese seeds showed a higher peak for SCT1 ($P=0.033$) only. No differences were observed on the protein-related structures of canola seeds from either country. On the other hand, all protein structures of canola meals presented differences when compared between countries ($P<0.05$). Yet, the principal component analysis was not able to completely differentiate the two countries in any of the protein or carbohydrate related structures analyzed.

Relationships between molecular structures and canola meals and seeds characteristics are not abundant in the literature. With this study, we could observe some relationships and develop prediction equations with significant R^2 and P values. For example, STCA seemed to be related to canola meals', while TCA was to canola seeds' parameters. Many parameters of canola seeds and some of canola meals appeared to be related to the amide region. Also, this chapter brought many prediction equations relating protein or carbohydrate structures to real performance aspects of canola seeds and meals.

In conclusion, the extensive amount of data and results generated and analyzed through this project points to affirming that: the canola seeds processed in Canada or in China are not different and mostly showed the same results, therefore the canola seeds exported to China are of

the same quality as the ones used internally in Canada; the meals produced both in Canada and in China are similar and that they perform similarly when fed to dairy cows; and that the ATR/FTIR technique is an efficient method to analyze protein and carbohydrate related structures and can be used with confidence to predict chemical, degradable and digestible aspects of canola seeds and meals.

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9. APPENDIX

A. 1. Correlation study between FTIR carbohydrate structures and the carbohydrate portions of the chemical profile of canola meals

	CHO (%DM)		NDF (%DM)		ADF (%DM)		ADL (%DM)		HEMI (%DM)		CEL (%DM)		ADL (%NDF)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.07	0.815	-0.51	0.053	-0.03	0.902	-0.43	0.113	-0.51	0.055	0.37	0.169	-0.04	0.899
TC2H	-0.15	0.593	-0.57	0.027	0.03	0.909	0.03	0.904	-0.54	0.036	-0.08	0.766	0.41	0.126
TC3H	-0.12	0.684	-0.52	0.049	-0.30	0.269	-0.33	0.225	-0.43	0.113	0.09	0.751	-0.01	0.960
TC4H	0.28	0.304	0.05	0.848	0.25	0.364	-0.38	0.163	-0.05	0.848	0.39	0.154	-0.35	0.195
CECH	0.34	0.214	0.23	0.410	0.05	0.849	-0.22	0.426	0.25	0.363	0.55	0.035	-0.41	0.132
STC1H	0.07	0.800	0.45	0.091	-0.22	0.433	-0.15	0.592	0.41	0.06	0.23	0.411	-0.46	0.085
STC2H	0.39	0.150	0.12	0.665	0.05	0.859	-0.13	0.651	0.12	0.665	0.45	0.094	-0.29	0.293
STC3H	0..40	0.141	-0.01	0.965	0.06	0.846	-0.20	0.464	-0.08	0.779	0.32	0.245	-0.26	0.345
STC4H	0.16	0.575	0.05	0.849	0.20	0.482	-0.24	0.382	-0.06	0.839	0.50	0.059	-0.30	0.279
TCA	-0.18	0.533	-0.31	0.260	-0.10	0.713	-0.46	0.081	-0.33	0.232	0.31	0.254	-0.25	0.362
CECA	0.21	0.459	0.41	0.128	-0.06	0.845	-0.13	0.548	0.48	0.071	0.38	0.168	-0.36	0.191
STCA	0.35	0.196	0.35	0.206	0.08	0.776	-0.35	0.206	0.31	0.260	0.64	0.011	-0.60	0.018
	ADF (%NDF)		NFC (%DM)		NFC (%CHO)		Starch (%NFC)		Sugar (%DM)		Sugar (%NFC)		NSC (%DM)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.52	0.046	0.42	0.117	0.44	0.099	-0.42	0.117	-0.25	0.371	-0.33	0.228	0.42	0.117
TC2H	0.48	0.070	0.44	0.103	0.45	0.091	-0.44	0.103	-0.48	0.069	-0.48	0.073	0.44	0.103
TC3H	0.37	0.171	0.36	0.190	0.41	0.134	-0.36	0.190	0.08	0.770	-0.13	0.638	0.36	0.190
TC4H	0.07	0.794	0.12	0.641	-0.01	0.964	-0.13	0.641	-0.18	0.525	-0.16	0.568	0.13	0.641
CECH	-0.18	0.518	0.12	0.684	-0.10	0.731	-0.12	0.684	0.08	0.775	0.08	0.785	0.15	0.684
STC1H	-0.41	0.134	-0.29	0.288	-0.29	0.288	0.29	0.288	0.00	1.000	0.06	0.839	-0.29	0.288
STC2H	-0.03	0.929	0.25	0.377	0.03	0.914	-0.25	0.377	0.10	0.731	-0.01	0.985	0.25	0.377
STC3H	0.16	0.569	0.19	0.492	0.06	0.819	-0.19	0.492	0.15	0.596	0.07	0.814	0.19	0.492
STC4H	0.09	0.737	-0.08	0.785	-0.16	0.580	0.08	0.785	0.16	0.567	0.15	0.584	-0.08	0.785
TCA	0.32	0.248	0.18	0.516	0.20	0.483	-0.18	0.516	-0.31	0.266	-0.31	0.260	0.18	0.516
CECA	-0.38	0.164	-0.12	0.666	-0.30	0.283	0.12	0.666	-0.21	0.443	-0.07	0.810	-0.12	0.666
STCA	-0.20	0.467	-0.08	0.781	-0.26	0.348	0.08	0.781	0.14	0.621	0.11	0.685	-0.08	0.781

DM: dry matter; CHO: carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL; acid detergent lignin; HEMI: hemicellulose; CEL: cellulose; NFC: non-fiber carbohydrate; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 2. Correlation study between FTIR carbohydrate structures and the carbohydrate portions of the chemical profile of canola seeds

	CHO (%DM)		NDF (%DM)		ADF (%DM)		ADL (%DM)		HEMI (%DM)		CEL (%DM)		ADL (%NDF)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.13	0.648	-0.32	0.240	-0.11	0.704	-0.20	0.478	-0.30	0.280	-0.09	0.761	0.10	0.723
TC2H	0.03	0.910	-0.56	0.031	-0.27	0.328	-0.24	0.394	-0.33	0.237	-0.27	0.334	0.13	0.639
TC3H	0.25	0.369	-0.26	0.348	-0.08	0.791	-0.32	0.240	-0.14	0.616	-0.08	0.781	0.02	0.950
TC4H	0.25	0.376	0.00	0.995	-0.25	0.369	-0.25	0.379	0.12	0.680	-0.34	0.221	-0.09	0.761
CECH	-0.07	0.800	-0.28	0.321	-0.21	0.462	-0.37	0.180	-0.05	0.847	-0.27	0.330	-0.06	0.829
STC1H	-0.14	0.625	0.07	0.800	0.39	0.152	0.66	0.008	-0.37	0.170	0.19	0.495	0.41	0.132
STC2H	-0.39	0.156	-0.21	0.447	0.10	0.713	0.08	0.785	-0.36	0.189	0.00	0.990	0.14	0.621
STC3H	-0.13	0.638	-0.32	0.252	-0.15	0.598	0.00	0.995	-0.28	0.322	-0.23	0.408	0.11	0.699
STC4H	-0.16	0.576	0.00	0.990	-0.11	0.704	-0.34	0.208	0.06	0.835	-0.11	0.695	-0.21	0.459
TCA	0.05	0.860	-0.33	0.231	-0.18	0.533	-0.42	0.119	-0.10	0.732	-0.15	0.603	-0.09	0.761
CECA	0.06	0.820	-0.53	0.044	0.00	0.990	0.00	0.990	-0.46	0.086	-0.11	0.704	0.31	0.254
STCA	-0.29	0.296	-0.34	0.221	0.11	0.695	0.11	0.699	-0.46	0.086	0.04	0.899	0.19	0.499
	ADF (%NDF)		NFC (%DM)		NFC (%CHO)		Sugar (%DM)		Sugar (%NFC)		NSC (%DM)			
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value		
TC1H	0.24	0.390	-0.04	0.889	0.18	0.524	0.47	0.076	0.23	0.405	-0.04	0.889		
TC2H	0.28	0.315	0.20	0.475	0.47	0.079	0.56	0.030	0.08	0.781	0.20	0.475		
TC3H	0.09	0.752	0.36	0.191	0.51	0.050	0.51	0.052	-0.11	0.685	0.36	0.191		
TC4H	-0.18	0.533	0.28	0.321	0.31	0.260	0.26	0.341	-0.10	0.713	0.28	0.321		
CECH	0.01	0.965	0.06	0.829	0.26	0.347	0.50	0.056	0.20	0.486	0.06	0.829		
STC1H	0.42	0.119	-0.25	0.365	-0.33	0.237	-0.58	0.022	-0.06	0.825	-0.25	0.365		
STC2H	0.35	0.201	-0.33	0.237	-0.15	0.594	0.14	0.630	0.38	0.160	-0.33	0.237		
STC3H	0.27	0.337	-0.06	0.835	0.13	0.643	0.17	0.545	0.15	0.584	-0.06	0.835		
STC4H	-0.08	0.771	-0.09	0.761	0.00	0.990	0.23	0.420	0.29	0.296	-0.09	0.761		
TCA	0.04	0.899	0.18	0.516	0.40	0.136	0.69	0.004	0.12	0.666	0.18	0.516		
CECA	0.42	0.121	0.23	0.420	0.47	0.079	0.34	0.221	-0.11	0.704	0.23	0.420		
STCA	0.47	0.079	-0.21	0.451	-0.01	0.980	0.17	0.550	0.32	0.248	-0.21	0.451		

DM: dry matter; CHO: carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL; acid detergent lignin; HEMI: hemicellulose; CEL: cellulose; NFC: non-fiber carbohydrate; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 3. Correlation study between FTIR carbohydrate structures and the carbohydrate portions of the CNCPS 6.5 of canola meals

	CA4 (%CHO)		CB1 (%CHO)		CB2 (%CHO)		CC (%CHO)		CB3 (%CHO)		RDCA4		RDCB2	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.26	0.357	0.04	0.879	0.37	0.169	-0.43	0.113	-0.37	0.181	-0.25	0.371	0.36	0.190
TC2H	-0.39	0.151	0.10	0.731	0.53	0.042	0.03	0.904	-0.56	0.029	-0.48	0.069	0.47	0.077
TC3H	0.08	0.770	0.13	0.645	0.29	0.300	-0.33	0.225	-0.36	0.194	0.08	0.770	0.25	0.374
TC4H	-0.25	0.361	-0.29	0.297	0.05	0.869	-0.38	0.163	0.05	0.874	-0.18	0.525	0.00	0.995
CECH	-0.04	0.884	-0.37	0.169	-0.12	0.679	-0.22	0.426	0.47	0.077	0.08	0.775	-0.05	0.854
STC1H	-0.07	0.809	-0.07	0.804	-0.15	0.601	-0.15	0.592	0.60	0.018	0.00	1.000	-0.04	0.899
STC2H	-0.07	0.809	-0.39	0.154	0.01	0.980	-0.13	0.651	0.31	0.260	0.10	0.731	0.09	0.751
STC3H	-0.01	0.965	-0.36	0.186	-0.08	0.779	-0.10	0.464	0.03	0.919	0.15	0.596	-0.03	0.914
STC4H	0.04	0.899	-0.15	0.607	-0.21	0.446	-0.24	0.382	0.20	0.470	0.16	0.567	-0.21	0.442
TCA	-0.34	0.221	0.16	0.565	0.26	0.348	-0.46	0.081	-0.19	0.508	-0.31	0.266	0.25	0.369
CECA	-0.29	0.302	-0.24	0.388	-0.06	0.840	-0.13	0.648	0.55	0.032	-0.21	0.443	0.04	0.880
STCA	-0.06	0.820	-0.34	0.219	-0.20	0.467	-0.35	0.206	0.50	0.056	0.14	0.621	-0.14	0.630
	RDCB3		Total RDC		RUCA4		RUCB2		RUCB3		RUCC		Total RUC	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.27	0.326	0.04	0.884	-0.25	0.379	0.35	0.167	-0.27	0.326	-0.44	0.104	-0.41	0.131
TC2H	-0.52	0.045	-0.24	0.394	-0.46	0.084	0.47	0.078	-0.52	0.045	0.01	0.970	-0.35	0.196
TC3H	-0.30	0.276	0.17	0.557	0.08	0.785	0.26	0.356	-0.30	0.276	-0.38	0.167	-0.42	0.120
TC4H	0.13	0.645	0.23	0.412	-0.19	0.507	0.01	0.964	0.13	0.645	-0.26	0.341	-0.04	0.889
CECH	0.51	0.052	0.55	0.033	0.05	0.851	-0.07	0.802	0.51	0.052	-0.12	0.665	0.27	0.332
STC1H	0.54	0.037	0.24	0.381	-0.02	0.939	-0.05	0.859	0.54	0.037	-0.16	0.566	0.23	0.411
STC2H	0.34	0.211	0.68	0.005	0.08	0.770	0.08	0.789	0.34	0.211	-0.06	0.844	0.24	0.388
STC3H	0.08	0.789	0.37	0.174	0.14	0.611	-0.04	0.889	0.08	0.789	-0.14	0.618	0.03	0.914
STC4H	0.26	0.344	0.26	0.419	0.14	0.619	-0.21	0.448	0.26	0.344	-0.25	0.375	-0.20	0.478
TCA	-0.11	0.695	-0.09	0.752	-0.31	0.256	0.25	0.376	-0.11	0.695	-0.52	0.046	-0.38	0.168
CECA	0.53	0.041	0.34	0.221	-0.28	0.416	0.02	0.945	0.53	0.04	-0.05	0.850	0.37	0.173
STCA	0.56	0.028	0.51	0.052	0.11	0.689	-0.15	0.593	0.56	0.028	-0.28	0.315	0.23	0.413

DM: dry matter; CHO: carbohydrate; RDCA4: ruminally degradable water-soluble carbohydrates; RDCB2: ruminally degradable soluble fiber; RDCB3: ruminally degradable digestible fiber; TotalRDC: total ruminally degradable carbohydrates; RUCA4: ruminally undegradable water soluble carbohydrates; RUCB2: ruminally undegradable soluble fiber; RUCB3: ruminally undegradable digestible fiber; RUCC: indigestible fiber; TotalRUC: total ruminally undegradable carbohydrates; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 4. Correlation study between FTIR carbohydrate structures and the carbohydrate portions of the CNCPS 6.5 of canola seeds

	CA4 (%CHO)		CB2 (%CHO)		CC (%CHO)		CB3 (%CHO)		RDCA4		RDCB2	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.26	0.348	-0.16	0.559	-0.20	0.475	-0.13	0.639	0.47	0.076	-0.24	0.383
TC2H	0.17	0.541	0.10	0.970	-0.23	0.405	-0.45	0.095	0.56	0.030	-0.06	0.830
TC3H	-0.01	0.970	-0.26	0.341	-0.32	0.248	-0.41	0.128	0.51	0.052	-0.16	0.559
TC4H	-0.09	0.761	-0.26	0.355	-0.24	0.390	-0.19	0.491	0.26	0.341	-0.12	0.676
CECH	0.22	0.423	-0.23	0.404	-0.36	0.192	-0.19	0.498	0.50	0.057	-0.25	0.378
STC1H	-0.15	0.589	0.56	0.030	0.65	0.008	-0.13	0.638	-0.58	0.022	0.39	0.153
STC2H	0.27	0.328	0.23	0.420	0.08	0.781	0.13	0.639	0.14	0.630	-0.01	0.970
STC3H	0.15	0.593	0.35	0.196	0.01	0.960	-0.13	0.648	0.17	0.545	0.20	0.466
STC4H	0.21	0.459	-0.43	0.108	-0.35	0.196	0.15	0.585	0.23	0.420	-0.45	0.095
TCA	0.22	0.435	-0.31	0.260	-0.41	0.128	-0.31	0.260	0.69	0.004	-0.26	0.341
CECA	-0.05	0.860	0.29	0.296	0.00	0.990	-0.50	0.056	0.34	0.221	0.20	0.475
STCA	0.26	0.341	0.33	0.232	0.11	0.685	-0.01	0.970	0.17	0.550	0.05	0.850

	RDCB3		Total RDC		RUCA4		RUCB2		RUCB3		RUCC		Total RUC	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.24	0.390	-0.20	0.467	0.49	0.067	-0.24	0.383	-0.24	0.390	-0.29	0.302	-0.38	0.166
TC2H	-0.46	0.081	0.02	0.940	0.56	0.029	-0.06	0.830	-0.46	0.081	-0.16	0.576	-0.34	0.216
TC3H	-0.17	0.541	-0.04	0.889	0.52	0.048	-0.16	0.559	-0.17	0.541	-0.03	0.920	-0.11	0.690
TC4H	0.03	0.920	-0.08	0.771	0.27	0.334	-0.12	0.676	0.03	0.920	-0.04	0.880	-0.01	0.975
CECH	-0.08	0.766	-0.17	0.549	0.50	0.055	-0.25	0.378	-0.08	0.766	-0.31	0.261	-0.29	0.297
STC1H	-0.13	0.638	0.12	0.680	-0.57	0.027	0.39	0.153	-0.13	0.638	0.18	0.516	0.13	0.640
STC2H	-0.10	0.713	-0.09	0.761	0.15	0.589	-0.01	0.970	-0.10	0.713	-0.40	0.136	-0.30	0.277
STC3H	-0.23	0.405	0.15	0.602	0.18	0.527	0.20	0.466	-0.23	0.405	-0.25	0.379	-0.19	0.494
STC4H	0.20	0.475	-0.40	0.140	0.24	0.393	-0.45	0.095	0.20	0.475	-0.40	0.136	-0.28	0.311
TCA	-0.16	0.576	-0.09	0.742	0.69	0.004	-0.26	0.341	-0.16	0.576	-0.22	0.428	-0.28	0.321
CECA	-0.51	0.052	0.22	0.428	0.34	0.220	0.20	0.475	-0.51	0.052	0.00	0.990	-0.16	0.558
STCA	-0.22	0.428	0.03	0.930	0.19	0.494	0.05	0.850	-0.22	0.428	-0.33	0.237	-0.31	0.262

DM: dry matter; CHO: carbohydrate; RDCA4: ruminally degradable water-soluble carbohydrates; RDCB2: ruminally degradable soluble fiber; RDCB3: ruminally degradable digestible fiber; TotalRDC: total ruminally degradable carbohydrates; RUCA4: ruminally undegradable water soluble carbohydrates; RUCB2: ruminally undegradable soluble fiber; RUCB3: ruminally undegradable digestible fiber; RUCC: indigestible fiber; TotalRUC: total ruminally undegradable carbohydrates; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 5. Correlation study between FTIR carbohydrate structures and the DVE/OEB System for canola meals

	BCP		EDCP		MREE		DVME		DVE	
	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.00	0.995	-0.06	0.844	-0.04	0.879	-0.04	0.879	0.02	0.955
TC2H	0.32	0.254	-0.34	0.218	-0.45	0.091	-0.45	0.091	0.43	0.109
TC3H	0.19	0.675	-0.07	0.809	-0.02	0.939	-0.02	0.939	0.27	0.339
TC4H	-0.20	0.475	0.09	0.764	0.28	0.311	0.28	0.311	-0.37	0.176
CECH	-0.02	0.934	0.25	0.370	0.43	0.111	0.43	0.111	0.03	0.914
STC1H	-0.32	0.241	0.40	0.138	0.49	0.131	0.49	0.131	-0.41	0.171
STC2H	-0.10	0.727	0.22	0.441	0.45	0.799	0.45	0.799	-0.07	0.477
STC3H	-0.17	0.534	0.17	0.534	0.41	0.556	0.41	0.556	-0.17	0.614
STC4H	0.06	0.835	-0.10	0.722	0.15	0.940	0.15	0.940	0.02	0.643
TCA	-0.23	0.413	0.19	0.499	0.24	0.376	0.24	0.376	-0.25	0.405
CECA	-0.34	0.216	0.44	0.105	0.46	0.148	0.46	0.148	-0.39	0.114
STCA	-0.25	0.376	0.34	0.216	0.62	0.014	0.62	0.014	-0.28	0.254
	OEB		DVBE		MREN		DVE FMV			
	r	P value	r	P value	r	P value	r	P value		
TC1H	0.02	0.945	-0.01	0.975	-0.03	0.919	0.02	0.947		
TC2H	-0.30	0.274	0.41	0.128	-0.31	0.245	0.43	0.111		
TC3H	-0.02	0.950	0.17	0.548	-0.04	0.879	0.25	0.363		
TC4H	0.06	0.824	-0.31	0.253	0.06	0.824	-0.35	0.197		
CECH	0.22	0.437	-0.11	0.707	0.24	0.381	0.02	0.937		
STC1H	0.37	0.171	-0.47	0.075	0.41	0.127	-0.40	0.138		
STC2H	0.20	0.477	-0.15	0.592	0.21	0.461	-0.06	0.824		
STC3H	0.14	0.614	-0.25	0.376	0.16	0.574	-0.15	0.602		
STC4H	-0.13	0.643	-0.01	0.960	-0.13	0.652	0.03	0.902		
TCA	0.23	0.405	-0.28	0.308	0.21	0.459	-0.24	0.383		
CECA	0.43	0.114	-0.45	0.095	0.45	0.095	-0.38	0.157		
STCA	0.31	0.254	-0.38	0.164	0.34	0.221	-0.27	0.331		

BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on the energy available; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman

A. 6. Correlation study between FTIR carbohydrate structures and the DVE/OEB System for canola seeds

	BCP		EDCP		MREE		DVME		DVE	
	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.20	0.483	0.01	0.960	-0.17	0.550	-0.17	0.550	-0.05	0.860
TC2H	0.08	0.771	0.12	0.676	0.01	0.960	0.01	0.960	0.02	0.940
TC3H	0.27	0.328	-0.17	0.550	0.14	0.612	0.14	0.612	0.21	0.443
TC4H	0.43	0.114	-0.32	0.243	0.13	0.657	0.13	0.657	0.31	0.260
CECH	0.31	0.255	-0.06	0.829	-0.16	0.562	-0.16	0.562	0.05	0.859
STC1H	-0.38	0.157	0.13	0.657	-0.19	0.503	-0.19	0.503	-0.38	0.164
STC2H	-0.03	0.920	0.28	0.308	-0.46	0.087	-0.46	0.087	-0.35	0.201
STC3H	-0.09	0.756	0.15	0.593	-0.20	0.474	-0.20	0.474	-0.25	0.365
STC4H	0.31	0.260	0.03	0.920	-0.26	0.348	-0.26	0.348	0.07	0.810
TCA	0.26	0.341	-0.01	0.970	0.01	0.980	0.01	0.980	0.14	0.630
CECA	-0.17	0.550	0.22	0.435	0.03	0.930	0.03	0.930	-0.13	0.657
STCA	-0.19	0.491	0.36	0.187	-0.36	0.187	-0.36	0.187	-0.40	0.136
	OEB		DVBE		MREN		DVE FMV			
	r	P value	r	P value	r	P value	r	P value		
TC1H	0.08	0.771	0.10	0.713	0.01	0.960	-0.03	0.929		
TC2H	-003	0.910	0.08	0.781	0.12	0.676	0.04	0.884		
TC3H	-0.30	0.283	0.26	0.355	-0.17	0.550	0.24	0.393		
TC4H	-0.33	0.237	0.39	0.148	-0.32	0.243	0.33	0.234		
CECH	0.00	0.990	0.23	0.408	-0.06	0.829	0.07	0.814		
STC1H	0.30	0.271	-0.44	0.100	0.13	0.657	-0.41	0.135		
STC2H	0.46	0.087	-0.16	0.567	0.28	0308	-0.35	0.195		
STC3H	0.26	0.358	-0.18	0.528	0.15	0.593	-0.28	0.315		
STC4H	0.14	0.630	0.25	0.362	0.03	0.920	0.09	0.761		
TCA	-0.13	0.648	0.24	0.383	-0.01	0.970	0.16	0.567		
CECA	-0.02	0.940	-0.15	0.603	0.22	0.435	-0.10	0.713		
STCA	0.46	0.084	-0.28	0.321	0.36	0.187	-0.42	0.119		

BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on the energy available; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance.; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 7. Correlation study between FTIR carbohydrate structures and the carbohydrate portions of the energy profile of canola meals

	TdNFC		TdNDF		TDN1x		DE1x		DEP3x		ME3x	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.42	0.117	0.05	0.864	0.51	0.054	0.46	0.083	0.46	0.088	0.48	0.070
TC2H	0.44	0.103	-0.43	0.112	0.22	0.439	0.17	0.557	0.19	0.509	0.17	0.547
TC3H	0.36	0.190	0.01	0.975	0.43	0.106	0.45	0.089	0.51	0.051	0.46	0.086
TC4H	0.13	0.641	0.396	0.150	0.06	0.843	0.02	0.934	0.00	0.990	0.03	0.903
CECH	0.15	0.684	0.40	0.136	-0.08	0.790	-0.06	0.819	0.02	0.934	-0.07	0.801
STC1H	-0.29	0.288	0.48	0.067	-0.16	0.566	-0.15	0.587	-0.11	0.687	-0.14	0.613
STC2H	0.25	0.377	0.29	0.299	-0.15	0.588	-0.14	0.614	-0.10	0.721	-0.15	0.597
STC3H	0.19	0.492	0.32	0.253	-0.14	0.627	-0.18	0.523	-0.14	0.631	-0.15	0.603
STC4H	-0.08	0.785	0.42	0.115	0.10	0.722	0.07	0.809	0.03	0.924	0.06	0.841
TCA	0.18	0.516	0.25	0.361	0.18	0.516	0.18	0.528	0.22	0.426	0.21	0.449
CECA	-0.12	0.666	0.39	0.151	-0.19	0.491	-0.19	0.490	-0.14	0.628	-0.19	0.505
STCA	-0.08	0.781	0.65	0.008	-0.18	0.516	-0.16	0.562	-0.10	0.712	-0.16	0.574
	MEp3x		NEm3x		Neg3x		Nel3x		EstMilk			
	r	P value	r	P value	r	P value	r	P value	r	P value		
TC1H	0.44	0.100	0.46	0.083	0.45	0.090	0.414	0.125	0.43	0.113		
TC2H	0.17	0.542	0.16	0.582	0.15	0.603	0.15	0.598	0.12	0.659		
TC3H	0.52	0.047	0.45	0.089	0.48	0.071	0.51	0.054	0.55	0.034		
TC4H	0.01	0.977	0.01	0.974	0.02	0.947	-0.06	0.844	-0.07	0.807		
CECH	-0.02	0.944	-0.08	0.778	-0.04	0.878	-0.03	0.903	0.05	0.865		
STC1H	-0.15	0.591	-0.13	0.640	-0.16	0.573	-0.14	0.620	-0.08	0.778		
STC2H	-0.11	0.692	-0.15	0.590	-0.12	0.671	-0.10	0.723	-0.05	0.860		
STC3H	-0.16	0.574	-0.16	0.562	-0.15	0.605	-0.16	0.560	-0.15	0.597		
STC4H	0.04	0.886	0.04	0.901	0.10	0.726	0.02	0.944	0.04	0.893		
TCA	0.21	0.457	0.19	0.501	0.18	0.522	0.15	0.594	0.18	0.534		
CECA	-0.19	0.490	-0.19	0.501	-0.20	0.465	-0.18	0.532	-0.14	0.613		
STCA	-0.13	0.656	-0.17	0.534	-0.13	0.646	-0.15	0.598	-0.07	0.794		

tdNDF: total digestible neutral detergent fiber; tdNFC: total digestible non-fiber carbohydrate; TDN_{1x}: total digestible nutrients at one time maintenance level; NELp3x: net energy for lactation at a productive level of intake three times the maintenance level; NEm3x: net energy for maintenance; NEg3x: net energy for gain; Dep3x: digestible energy at a productive level of intake three times the maintenance level; FMV: feed milk value; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 8. Correlation study between FTIR carbohydrate structures and the energy profile of canola seeds

	TdNFC		TdNDF		TDN1x		DE1x		DEP3x		ME3x	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.04	0.889	-0.22	0.442	0.07	0.800	0.08	0.771	0.08	0.766	0.09	0.751
TC2H	0.20	0.475	-0.41	0.128	-0.04	0.899	-0.03	0.930	-0.02	0.955	-0.01	0.970
TC3H	0.36	0.191	-0.11	0.680	-0.32	0.243	-0.31	0.262	-0.30	0.273	-0.30	0.285
TC4H	0.28	0.321	0.08	0.785	-0.32	0.248	-0.30	0.277	-0.30	0.283	-0.30	0.276
CECH	0.06	0.829	-0.08	0.770	0.00	0.990	0.01	0.960	0.02	0.944	0.02	0.957
STC1H	-0.25	0.365	-0.21	0.454	0.22	0.424	0.23	0.419	0.21	0.458	0.20	0.465
STC2H	-0.33	0.237	-0.20	0.470	0.37	0.177	0.39	0.155	0.38	0.168	0.37	0.178
STC3H	-0.06	0.835	-0.20	0.472	0.19	0.499	0.20	0.478	0.19	0.496	0.17	0.546
STC4H	-0.09	0.761	0.15	0.597	0.02	0.950	0.04	0.899	0.04	0.889	0.04	0.894
TCA	0.18	0.516	-0.10	0.732	-0.11	0.704	-0.10	0.732	-0.09	0.746	-0.09	0.756
CECA	0.23	0.420	-0.54	0.038	-0.04	0.889	-0.03	0.930	-0.02	0.955	-0.01	0.985
STCA	-0.21	0.451	-0.28	0.304	0.32	0.243	0.33	0.229	0.32	0.247	0.31	0.261
	MEp3x		NEm3x		Neg3x		Nel3x		FMV			
	r	P value	r	P value	r	P value	r	P value	r	P value		
TC1H	0.08	0.770	0.07	0.800	0.08	0.780	0.10	0.736	0.11	0.694		
TC2H	-0.03	0.909	-0.04	0.889	-0.02	0.939	-0.02	0.939	0.00	0.995		
TC3H	-0.32	0.247	-0.33	0.236	-0.31	0.261	-0.31	0.260	-0.29	0.3011		
TC4H	-0.31	0.264	-0.34	0.223	-0.30	0.276	-0.30	0.284	-0.26	0.340		
CECH	0.01	0.975	0.02	0.944	0.01	0.965	0.02	0.934	0.05	0.851		
STC1H	0.20	0.477	0.21	0.462	0.22	0.434	0.19	0.489	0.21	0.454		
STC2H	0.37	0.171	0.34	0.210	0.38	0.16	0.38	0.158	0.42	0.122		
STC3H	0.17	0.539	0.15	0.593	0.19	0.510	0.18	0.524	0.21	0.464		
STC4H	0.05	0.869	0.01	0.965	0.05	0.874	0.06	0.844	0.08	0.785		
TCA	-0.10	0.732	-0.11	0.700	-0.10	0.732	-0.09	0.760	-0.07	0.805		
CECA	-0.04	0.879	-0.05	0.874	-0.02	0.945	-0.03	0.914	0.00	1.000		
STCA	0.30	0.270	0.30	0.286	0.33	0.236	0.31	0.255	0.34	0.215		

tdNDF: total digestible neutral detergent fiber; tdNFC: total digestible non-fiber carbohydrate; TDN_{1x}: total digestible nutrients at one time maintenance level; NELp3x: net energy for lactation at a productive level of intake three times the maintenance level; NEm3x: net energy for maintenance; NEg3x: net energy for gain; Dep3x: digestible energy at a productive level of intake three times the maintenance level; FMV: feed milk value; TC: total carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman.

A. 9. Correlation study between FTIR carbohydrate structures and the NRC System for canola meals

	MCP_RDP		MCP_TDN		AMCP		RUP		ARUP	
AECP	MP	DPB	NR	P value	r	P value	r	P value	r	P value
TC1H	-0.06	0.844	0.51	0.054	0.49	0.064	0.00	0.995	-0.01	0.975
TC2H	-0.34	0.218	0.22	0.439	0.20	0.470	0.32	0.254	0.41	0.128
TC3H	-0.07	0.809	0.43	0.106	0.43	0.109	0.12	0.675	0.17	0.548
TC4H	0.09	0.764	0.06	0.843	0.03	0.914	-0.20	0.475	-0.32	0.253
CECH	0.45	0.370	-0.08	0.790	-0.08	0.767	-0.02	0.934	-0.11	0.707
STC1H	0.40	0.138	-0.16	0.566	-0.15	0.592	-0.32	0.241	-0.47	0.075
STC2H	0.22	0.441	-0.15	0.588	-0.16	0.581	-0.10	0.727	-0.15	0.592
STC3H	0.17	0.534	-0.14	0.627	-0.15	0.587	-0.17	0.534	-0.25	0.376
STC4H	-0.10	0.722	0.10	0.722	0.08	0.780	0.06	0.835	-0.01	0.960
TCA	0.19	0.499	0.18	0.516	0.16	0.571	-0.23	0.413	-0.28	0.308
CECA	0.44	0.105	-0.19	0.491	-0.20	0.487	-0.34	0.216	-0.45	0.095
STCA	0.34	0.216	-0.18	0.516	-0.20	0.478	-0.25	0.376	-0.38	0.164
	AECP		MP		DPB		NRC FMV		ECP	
	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	-0.15	0.604	0.04	0.889	-0.11	0.708	0.004	0.889	-0.19	0.510
TC2H	0.41	0.130	0.45	0.094	-0.36	0.184	0.45	0.094	0.38	0.167
TC3H	-0.27	0.340	0.26	0.346	-0.09	0.761	0.26	0.346	-0.32	0.248
TC4H	-0.32	0.254	-0.37	0.180	0.14	0.618	-0.37	0.180	-0.30	0.286
CECH	-0.40	0.141	-0.12	0.670	0.25	0.360	-0.12	0.670	-0.41	0.134
STC1H	-0.50	0.059	-0.50	0.057	0.38	0.167	-0.50	0.057	-0.48	0.069
STC2H	-0.58	0.024	-0.20	0.469	0.23	0.410	-0.20	0.469	-0.58	0.025
STC3H	-0.80	<0.001	-0.31	0.254	0.20	0.480	-0.31	0.254	-0.80	<0.001
STC4H	-0.75	0.001	-0.10	0.737	-0.04	0.879	-0.10	0.737	-0.75	0.001
TCA	-0.35	0.200	-0.26	0.348	0.16	0.559	-0.26	0.348	-0.35	0.205
CECA	-0.31	0.274	-0.49	0.062	0.42	0.118	-0.49	0.062	-0.30	0.286
STCA	-0.86	<0.001	-0.42	0.121	0.37	0.173	-0.42	0.121	-0.85	<0.001

MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN_{3x}; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; ECP: endogenous protein in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; TC: total

carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman

A. 10. Correlation study between FTIR carbohydrate structures and the NRC System for canola seeds

	MCP_RDP		MCP_TDN		AMCP		RUP		ARUP	
AECP	MP	DPB	NR	P value	r	P value	r	P value	r	P value
TC1H	0.01	0.960	0.07	0.800	0.07	0.800	0.20	0.483	0.10	0.713
TC2H	0.12	0.676	-0.04	0.899	-0.04	0.899	0.08	0.771	0.08	0.781
TC3H	-0.17	0.550	-0.32	0.243	-0.32	0.243	0.27	0.328	0.26	0.355
TC4H	-0.32	0.243	-0.32	0.248	-0.32	0.248	0.43	0.114	0.39	0.148
CECH	-0.06	0.829	0.00	0.990	0.00	0.990	0.31	0.255	0.23	0.408
STC1H	0.13	0.657	0.22	0.424	0.22	0.424	-0.38	0.157	-0.44	0.100
STC2H	0.28	0.308	0.37	0.177	0.37	0.177	-0.03	0.920	-0.16	0.567
STC3H	0.15	0.593	0.19	0.500	0.19	0.500	-0.09	0.756	-0.18	0.528
STC4H	0.03	0.920	0.02	0.950	0.02	0.950	0.31	0.260	0.25	0.362
TCA	-0.01	0.970	-0.11	0.704	-0.11	0.704	0.26	0.341	0.24	0.383
CECA	0.22	0.435	-0.04	0.889	-0.04	0.889	-0.17	0.550	-0.15	0.603
STCA	0.36	0.187	0.32	0.243	0.32	0.243	-0.19	0.491	-0.28	0.321
	AECP		MP		DPB		NRC FMV		ECP	
	r	P value	r	P value	r	P value	r	P value	r	P value
TC1H	0.46	0.086	0.10	0.723	-0.06	0.820	0.09	0.761	0.49	0.061
TC2H	0.57	0.027	-0.05	0.870	0.13	0.643	-0.06	0.820	0.60	0.019
TC3H	0.73	0.002	-0.06	0.820	0.16	0.563	-0.06	0.825	0.74	0.002
TC4H	0.56	0.029	0.15	0.603	0.08	0.781	0.16	0.571	0.57	0.026
CECH	0.72	0.002	0.12	0.661	-0.05	0.869	0.13	0.642	0.75	0.001
STC1H	-0.96	<0.001	-0.15	0.602	-0.03	0.907	-0.15	0.582	-0.96	<0.001
STC2H	-0.04	0.889	0.08	0.781	-0.09	0.742	0.08	0.785	0.01	0.965
STC3H	-0.08	0.777	-0.08	0.781	0.10	0.713	-0.08	0.790	-0.05	0.857
STC4H	0.55	0.032	0.30	0.277	-0.07	0.810	0.31	0.253	0.58	0.024
TCA	0.79	<0.001	0.01	0.960	0.06	0.835	0.01	0.975	0.81	<0.001
CECA	0.38	0.166	-0.28	0.321	0.21	0.458	-0.30	0.279	0.41	0.128
STCA	0.13	0.646	-0.07	0.800	0.05	0.874	-0.08	0.790	-0.09	0.761

MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN_{3x}; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; ECP: endogenous protein in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; TC: total

carbohydrate; STC: structural carbohydrate; A: area; H: height; 1, 2, 3, 4: refers to peaks; r: correlation coefficient using Spearman

A. 11. Correlation study between FTIR protein structures and the protein portions of the chemical profile of canola meals

	CP(%DM)		SCP (%CP)		SCP (%DM)		ADIP (%CP)		ADIP (%DM)		NDIP (%CP)		NDIP (%DM)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.06	0.686	0.51	<0.001	0.48	0.001	-0.26	0.087	-0.29	0.057	-0.52	<0.001	-0.54	<0.001
A1	0.37	0.012	0.05	0.732	0.09	0.566	0.20	0.198	0.25	0.100	-0.06	0.690	-0.03	0.823
A2	0.26	0.083	-0.11	0.495	-0.07	0.642	0.28	0.068	0.33	0.029	0.06	0.712	0.07	0.663
Area ratio	0.03	0.857	0.40	0.007	0.39	0.009	-0.29	0.056	-0.33	0.027	-0.35	0.017	-0.35	0.020
A1H	0.36	0.015	0.16	0.293	0.18	0.230	0.10	0.520	0.15	0.340	-0.15	0.312	-0.13	0.384
A2H	0.16	0.286	-0.20	0.190	-0.18	0.238	0.28	0.068	0.32	0.031	0.11	0.469	0.11	0.468
Height	0.02	0.911	0.53	<0.001	0.51	<0.001	-0.43	0.004	-0.47	0.001	-0.44	0.003	-0.44	0.003
Alpha	0.42	0.004	0.19	0.208	0.22	0.146	0.047	0.759	0.10	0.528	-0.20	0.185	-0.18	0.242
Beta	0.31	0.038	0.03	0.822	0.07	0.663	-0.14	0.349	0.19	0.218	-0.10	0.534	-0.07	0.631
Ratio	0.35	0.018	0.30	0.044	0.31	0.037	-0.18	0.249	-0.14	0.344	-0.32	0.033	-0.30	0.045

DM: dry matter; CP: crude protein; SCP: soluble crude protein; ADIP: acid detergent-insoluble crude protein; NDIP: neutral detergent-insoluble crude protein; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 12. Correlation study between FTIR protein structures and the protein portions of the chemical profile of canola seeds

	CP(%DM)		SCP (%CP)		SCP (%DM)		ADIP (%CP)		ADIP (%DM)		NDIP (%CP)		NDIP (%DM)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.25	0.097	0.64	<0.001	0.64	<0.001	0.17	0.264	0.09	0.548	-0.38	0.010	-0.42	0.004
A1	0.34	0.023	-0.22	0.142	-0.19	0.204	-0.29	0.058	-0.21	0.175	-0.33	0.030	-0.24	0.111
A2	0.36	0.015	-0.39	0.009	-0.39	0.015	-0.28	0.060	-0.20	0.199	-0.20	0.191	-0.12	0.448
Area ratio	-0.35	0.019	-0.52	<0.001	0.52	<0.001	0.27	0.072	0.18	0.230	0.03	0.824	-0.04	0.805
A1H	0.24	0.119	-0.06	0.680	-0.06	0.813	-0.23	0.134	-0.17	0.253	-0.33	0.025	-0.27	0.075
A2H	0.49	<0.001	-0.48	<0.001	-0.48	0.003	-0.38	0.011	-0.27	0.078	-0.10	0.497	0.002	0.990
Height	-0.43	0.003	0.62	<0.001	0.61	<0.001	0.41	0.006	0.30	0.042	-0.06	0.674	-0.15	0.326
Alpha	0.30	0.046	-0.21	0.166	-0.21	0.228	-0.29	0.053	-0.22	0.146	-0.26	0.088	-0.19	0.216
Beta	0.35	0.017	-0.22	0.142	-0.22	0.208	-0.25	0.095	-0.17	0.278	-0.26	0.088	-0.17	0.253
Ratio	-0.10	0.507	0.00	0.987	0.00	0.934	-0.07	0.628	-0.12	0.444	0.00	0.998	-0.03	0.832

DM: dry matter; CP: crude protein; SCP: soluble crude protein; ADIP: acid detergent-insoluble crude protein; NDIP: neutral detergent-insoluble crude protein; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 13. Correlation study between FTIR protein structures and the protein portions of the CNCPS Profile of canola meals

	(%CP)										(%TP)					
	PA2		PC		PB2		PB1		TP		PA2		PB1		PB2	
	R	P value	r	P value	r	P value	R	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.19	0.491	-0.08	0.771	-0.43	0.111	0.07	0.810	0.08	0.771	0.19	0.491	0.03	0.910	-0.45	0.092
A1	0.27	0.328	-0.15	0.594	-0.38	0.160	-0.01	0.960	0.15	0.594	0.27	0.328	-0.05	0.850	-0.42	0.121
A2	0.04	0.880	-0.09	0.761	-0.52	0.046	0.25	0.365	0.09	0.761	0.04	0.880	0.22	0.428	-0.55	0.034
Area ratio	0.34	0.216	-0.03	0.909	0.39	0.147	-0.57	0.025	0.03	0.909	0.34	0.216	-0.57	0.028	0.37	0.170
A1H	0.25	0.369	-0.25	0.369	-0.41	0.125	0.05	0.864	0.25	0.369	0.25	0.369	0.01	0.970	-0.45	0.095
A2H	-0.20	0.470	-0.04	0.889	-0.44	0.102	0.44	0.104	0.04	0.889	-0.20	0.470	0.46	0.086	-0.46	0.085
Height	0.48	0.069	-0.19	0.508	0.01	0.970	-0.44	0.103	0.19	0.508	0.48	0.069	-0.53	0.041	-0.03	0.910
Alpha	0.11	0.708	-0.24	0.394	-0.43	0.114	0.20	0.470	0.24	0.394	0.11	0.708	0.17	0.537	-0.45	0.089
Beta	0.36	0.187	-0.13	0.657	-0.49	0.064	-0.03	0.914	0.13	0.657	0.36	0.187	-0.07	0.800	-0.51	0.050
Ratio	-0.12	0.666	-0.13	0.638	0.02	0.934	0.28	0.309	0.13	0.638	-0.12	0.666	0.30	0.271	0.01	0.965

	Rumen Degradable fractions (%DM)								**Rumen Undegradable fractions (%DM)**									
	PA2		PB1		PB2		TOTAL RDP		PA2		PB1		PB2		PC		TOTAL RUP	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
	0.17	0.545	0.07	0.810	-0.41	0.126	0.21	0.451	0.17	0.541	0.07	0.810	-0.42	0.123	-0.04	0.899	-0.18	0.524
	0.25	0.368	0.02	0.960	-0.37	0.181	0.29	0.290	0.25	0.372	0.01	0.960	-0.37	0.177	-0.10	0.713	-0.15	0.594
	0.03	0.930	0.24	0.390	-0.52	0.049	0.09	0.742	0.03	0.914	0.24	0.39N	-0.52	0.048	-0.05	0.860	-0.20	0.475
	0.33	0.228	-0.46	0.084	0.42	0.121	0.27	0.331	0.32	0.244	-0.46	0.084	0.41	0.128	-0.00	0.990	0.20	0.483
	0.22	0.427	0.07	0.810	-0.40	0.141	0.28	0.321	0.22	0.431	0.07	0.810	-0.40	0.139	-0.21	0.459	-0.23	0.413
	-0.20	0.474	0.30	0.286	-0.44	0.099	-0.14	0.620	-0.20	0.467	0.30	0.286	-0.44	0.097	-0.00	0.990	-0.16	0.567
	0.45	0.094	-0.18	0.533	0.04	0.884	0.44	0.105	0.45	0.090	-0.18	0.533	0.03	0.914	-0.18	0.516	0.03	0.930
	0.09	0.761	0.21	0.462	-0.42	0.125	0.17	0.545	0.09	0.749	0.21	0.462	-0.42	0.123	-0.18	0.511	-0.22	0.431
	0.33	0.226	-0.06	0.840	-0.47	0.074	0.32	0.248	0.33	0.228	-0.06	0.840	-0.48	0.073	-0.10	0.733	-0.26	0.341
	-0.10	0.713	0.21	0.462	0.01	0.965	0.02	0.934	-0.10	0.729	0.21	0.462	0.02	0.945	-0.06	0.830	-0.02	0.945

DM: dry matter; CP: crude protein; PA2: soluble true protein; PB1: moderately degradable protein; PB2: slowly degradable protein; PC: unavailable crude protein; Total RDP: total rumen degradable protein; Total RUP: total rumen undegradable protein. r: correlation coefficient using Spearman.

A. 14. Correlation study between FTIR protein structures and the protein portions of the CNCPS Profile of canola seeds

	(%CP)										(%TP)					
	PA2		PC		PB2		PB1		TP		PA2		PB1		PB2	
	R	P value	r	P value	r	P value	R	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.74	0.002	-0.32	0.248	-0.43	0.108	-0.63	0.012	0.32	0.248	0.75	0.001	-0.63	0.012	-0.43	0.108
A1	0.76	0.001	-0.26	0.351	-0.50	0.060	-0.64	0.010	0.26	0.351	0.78	0.001	-0.64	0.010	-0.50	0.060
A2	0.56	0.030	-0.45	0.097	-0.24	0.383	-0.45	0.090	0.45	0.097	0.58	0.025	-0.45	0.090	-0.24	0.383
Area ratio	0.15	0.594	0.38	0.168	-0.11	0.685	-0.17	0.550	-0.38	0.168	0.14	0.612	-0.17	0.550	-0.11	0.685
A1H	0.65	0.008	-0.32	0.240	-0.32	0.247	-0.56	0.030	0.32	0.240	0.66	0.007	-0.56	0.030	-0.32	0.247
A2H	0.57	0.028	-0.49	0.061	-0.06	0.845	-0.50	0.056	0.49	0.061	0.57	0.027	-0.50	0.056	-0.06	0.845
Height	-0.11	0.695	0.58	0.023	-0.50	0.060	0.11	0.695	-0.58	0.023	-0.10	0.713	0.11	0.695	-0.50	0.060
Alpha	0.74	0.002	-0.25	0.379	-0.13	0.648	-0.69	0.004	0.25	0.379	0.73	0.002	-0.69	0.004	-0.13	0.648
Beta	0.55	0.034	-0.32	0.239	-0.38	0.161	-0.46	0.085	0.32	0.239	0.56	0.029	-0.50	0.085	-0.38	0.161
Ratio	0.46	0.084	-0.32	0.251	0.33	0.237	-0.51	0.054	0.32	0.251	0.43	0.111	-0.51	0.054	0.33	0.237

	Rumen Degradable fractions (%DM)								Rumen Undegradable fractions (%DM)									
	PA2		PB1		PB2		TOTAL RDP		PA2		PB1		PB2		PC		TOTAL RUP	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
	0.75	0.001	-0.64	0.010	-0.44	0.103	0.76	0.001	0.75	0.001	-0.64	0.001	-0.43	0.108	-0.39	0.153	-0.74	0.002
	0.76	0.001	-0.65	0.009	-0.50	0.057	0.75	0.001	0.76	0.001	-0.65	0.001	-0.50	0.060	-0.33	0.234	-0.78	<0.001
	0.59	0.021	-0.48	0.074	-0.25	0.368	0.60	0.017	0.59	0.021	-0.48	0.021	-0.24	0.383	-0.50	0.055	-0.59	0.022
	0.11	0.685	-0.15	0.603	-0.11	0.690	0.06	0.830	0.11	0.685	-0.15	0.685	-0.11	0.685	0.40	0.135	-0.13	0.639
	0.67	0.006	-0.57	0.026	-0.32	0.240	0.69	0.004	0.67	0.006	-0.57	0.026	-0.32	0.247	-0.39	0.153	-0.65	0.009
	0.60	0.019	-0.52	0.047	-0.06	0.827	0.64	0.010	0.60	0.019	-0.52	0.047	-0.06	0.845	-0.53	0.041	-0.59	0.021
	-0.14	0.612	0.14	0.621	-0.49	0.062	-0.22	0.439	-0.14	0.612	0.14	0.621	-0.50	0.060	0.56	0.031	0.17	0.541
	0.76	0.001	-0.68	0.005	-0.13	0.634	0.79	<0.001	0.76	0.001	-0.68	0.005	-0.13	0.648	-0.28	0.310	-0.69	0.005
	0.55	0.032	-0.48	0.072	-0.39	0.153	0.56	0.030	0.55	0.032	-0.48	0.072	-0.38	0.161	-0.39	0.146	-0.56	0.029
	0.49	0.062	-0.48	0.071	0.32	0.242	0.57	0.027	0.49	0.062	-0.48	0.071	0.33	0.237	-0.32	0.253	-0.41	0.132

DM: dry matter; CP: crude protein; PA2: soluble true protein; PB1: moderately degradable protein; PB2: slowly degradable protein; PC: unavailable crude protein; Total RDP: total rumen degradable protein; Total RUP: total rumen undegradable protein. r: correlation coefficient using Spearman.

A. 15. Correlation study between FTIR protein structures of canola meals and the DVE/OEB system

	MREE		DVME		DVE		OEB		DVBE		MREN		DVE FMV	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.02	0.950	-0.02	0.950	0.33	0.227	-0.14	0.630	0.26	0.355	-0.11	0.685	0.32	0.242
A1	0.07	0.810	0.07	0.810	0.27	0.334	-0.05	0.870	0.18	0.524	-0.02	0.940	0.26	0.358
A2	-0.13	0.657	-0.13	0.657	0.43	0.114	-0.18	0.516	0.36	0.191	-0.18	0.516	0.41	0.124
Area ratio	0.26	0.358	0.26	0.358	-0.41	0.126	0.34	0.221	-0.41	0.134	0.36	0.182	-0.43	0.110
A1H	0.03	0.930	0.03	0.930	0.22	0.443	-0.01	0.960	0.14	0.612	0.00	0.990	0.20	0.474
A2H	-0.31	0.259	-0.31	0.259	0.57	0.026	-0.27	0.327	0.50	0.056	-0.29	0.289	0.56	0.028
Height	0.32	0.243	0.32	0.243	-0.44	0.098	0.32	0.248	-0.44	0.101	0.36	0.182	-0.46	0.086
Alpha	-0.08	0.785	-0.08	0.785	0.30	0.271	-0.11	0.704	0.25	0.372	-0.10	0.723	0.29	0.293
Beta	0.05	0.870	0.05	0.870	0.25	0.362	-0.06	0.820	0.17	0.541	-0.05	0.860	0.24	0.386
Ratio	-0.13	0.647	-0.13	0.647	0.18	0.515	-0.05	0.869	0.20	0.478	-0.08	0.776	0.17	0.534

MREE: microbial protein synthesized in the rumen based on the energy available; DVME: rumen synthesized microbial protein digested in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance; DVBE: truly absorbed bypass protein in the small intestine; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVE FMV: estimated milk production based on the DVE system in kg milk/kg DM feed. Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 16. Correlation study between FTIR protein structures of canola seeds and the DVE/OEB system

	MREE		DVME		DVE		OEB		DVBE		MREN		DVE FMV	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.21	0.451	0.21	0.451	-0.04	0.899	-0.20	0.475	-0.15	0.594	-0.02	0.940	-0.04	0.899
A1	0.25	0.362	0.25	0.362	-0.05	0.850	-0.24	0.383	-0.21	0.451	-0.06	0.840	-0.06	0.844
A2	0.09	0.742	0.09	0.742	0.01	0.980	-0.13	0.639	-0.01	0.970	-0.04	0.880	0.01	0.970
Area ratio	0.36	0.191	0.36	0.191	0.08	0.781	-0.28	0.321	-0.18	0.533	-0.03	0.930	0.07	0.810
A1H	0.18	0.532	0.18	0.532	-0.01	0.985	-0.22	0.430	-0.04	0.889	-0.15	0.5593	-0.01	0.975
A2H	0.21	0.462	0.21	0.46	0.20	0.470	-0.26	0.354	0.17	0.545	-0.06	0.845	0.21	0.458
Height	-0.26	0.348	-0.26	0.348	-0.45	0.095	0.20	0.483	-0.44	0.105	-0.13	0.648	-0.45	0.095
Alpha	0.36	0.184	0.36	0.184	0.23	0.408	-0.44	0.104	0.16	0.580	-0.23	0.412	0.23	0.406
Beta	0.09	0.761	0.09	0.761	-0.13	0.634	-0.05	0.854	-0.20	0.478	0.03	0.904	-0.15	0.601
Ratio	0.31	0.260	0.31	0.260	0.43	0.108	-0.49	0.066	0.48	0.069	-0.44	0.101	0.44	0.100

MREE: microbial protein synthesized in the rumen based on the energy available; DVME: rumen synthesized microbial protein digested in the small intestine; DVE: truly digested protein in the small intestine; OEB: degradable protein balance; DVBE: truly absorbed bypass protein in the small intestine; MREN: microbial protein synthesized in the rumen based on available rumen degradable protein; DVE FMV: estimated milk production based on the DVE system in kg milk/kg DM feed. Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 17. Correlation study between FTIR protein structures and the energy profile of canola meals

	TDCP		TDN_{1x}		DE_{1x}		DEP_{3x}		ME_{3x}	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.09	0.540	-0.24	0.117	-0.22	0.142	-0.20	0.199	-0.22	0.156
A1	0.32	0.033	0.39	0.009	0.45	0.002	0.46	0.002	0.46	0.002
A2	0.20	0.180	0.29	0.057	0.35	0.020	0.34	0.023	0.35	0.020
Area ratio	0.07	0.638	-0.12	0.420	-0.15	0.342	-0.12	0.438	-0.15	0.340
A1H	0.32	0.034	0.35	0.018	0.42	0.004	0.43	0.003	0.43	0.004
A2H	0.11	0.487	0.28	0.065	0.32	0.030	0.32	0.033	0.32	0.030
Height	0.09	0.575	-0.20	0.185	-0.22	0.151	-0.20	0.197	-0.22	0.147
Alpha	0.38	0.010	0.41	0.006	0.47	0.001	0.47	0.001	0.47	0.001
Beta	0.27	0.079	044	0.003	0.50	0.001	0.50	0.001	0.50	<0.001
Ratio	0.36	0.016	0.12	0.424	0.15	0.330	0.16	0.285	0.15	0.341

	MEP_{3x}		NEM_{3x}		NEG_{3x}		$NELP_{3x}$	
	r	P value	r	P value	r	P value	r	P value
Peak area	-0.18	0.236	-0.21	0.167	-0.23	0.133	-0.19	0.220
A1	0.45	0.002	0.44	0.002	0.43	0.004	0.46	0.002
A2	0.33	0.027	0.33	0.027	0.32	0.033	0.34	0.022
Area ratio	-0.11	0.480	-0.13	0.387	-0.13	0.392	-0.10	0.506
A1H	0.42	0.004	0.42	0.005	0.39	0.007	0.42	0.004
A2H	0.30	0.046	0.31	0.037	0.30	0.045	0.30	0.049
Height	-0.17	0.255	-0.20	0.180	-0.20	0.184	-0.17	0.278
Alpha	0.48	0.001	0.46	0.001	0.45	0.002	0.48	0.001
Beta	0.48	0.001	0.49	0.001	0.47	0.001	0.48	0.001
Ratio	0.19	0.219	0.16	0.308	0.16	0.282	0.21	0.160

DM: dry matter; CP: crude protein; TDCP: Total digestible CP; TDN_{1x}: total digestible nutrients at one time maintenance level; $DE_{1x:}$: digestible energy at a one time maintenance level; DE_{p3x}: digestible energy at a productive level of intake three times the maintenance level; ME_{3x}: metabolizable energy for gain at three times the maintenance level; ME_{p3x}: metabolizable energy at a productive level of intake three times the maintenance level; NEm_{3x}: net energy for maintenance; NEg_{3x}: net energy for gain; NEL_{p3x}: net energy for lactation at a productive level of intake three times the maintenance level; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 18. Correlation study between FTIR protein structures and the energy profile of canola seeds

	TDCP		TDN_{1x}		DE_{1x}		DEP_{3x}		ME_{3x}	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.27	0.078	0.23	0.121	0.23	0.128	0.21	0.158	0.22	0.143
A1	0.35	0.018	-0.19	0.218	-0.16	0.294	-0.16	0.302	-0.16	0.302
A2	0.37	0.011	-0.27	0.071	-0.25	0.105	-0.24	0.115	-0.25	0.105
Area ratio	-0.37	0.013	0.25	0.100	0.22	0.141	0.21	0.161	0.22	0.141
A1H	0.24	0.119	-0.11	0.481	-0.08	0.581	-0.08	0.587	-0.09	0.580
A2H	0.50	0.001	-0.26	0.080	-0.23	0.133	-0.22	0.149	-0.23	0.133
Height	-0.46	0.002	0.29	0.051	0.26	0.082	0.25	0.097	0.26	0.082
Alpha	0.31	0.040	-0.21	0.176	-0.18	0.247	-0.17	0.256	-0.17	0.252
Beta	0.35	0.018	-0.20	0.196	-0.17	0.265	-0.16	0.283	-0.17	0.272
Ratio	-0.09	0.562	-0.10	0.520	-0.09	0.549	-0.10	0.524	-0.10	0.531
	MEP_{3x}		NEM_{3x}		NEG_{3x}		$NELP_{3x}$			
	r	P value	r	P value	r	P value	r	P value		
Peak area	0.23	0.128	0.22	0.139	0.22	0.140	0.26	0.086		
A1	-0.20	0.192	-0.16	0.281	-0.16	0.275	-0.21	0.167		
A2	-0.29	0.056	-0.25	0.092	-0.25	0.094	-0.30	0.046		
Area ratio	0.26	0.082	0.23	0.128	0.23	0.130	0.28	0.064		
A1H	-0.12	0.440	-0.10	0.512	-0.09	0.551	-0.12	0.429		
A2H	-0.27	0.074	-0.23	0.121	-0.24	0.120	-0.28	0.058		
Height	0.30	0.048	0.27	0.079	0.27	0.075	0.32	0.032		
Alpha	-0.21	0.157	-0.19	0.218	-0.18	0.232	-0.22	0.141		
Beta	-020	0.186	-0.18	0.233	-0.17	0.256	-0.21	0.160		
Ratio	-0.12	0.436	-0.10	0.523	-0.11	0.490	-0.11	0.477		

DM: dry matter; CP: crude protein; TDCP: Total digestible CP; TDN_{1x}: total digestible nutrients at one time maintenance level; $DE_{1x:}$: digestible energy at a one time maintenance level; DE_{p3x}: digestible energy at a productive level of intake three times the maintenance level; ME_{3x}: metabolizable energy for gain at three times the maintenance level; ME_{p3x}: metabolizable energy at a productive level of intake three times the maintenance level; NEm_{3x}: net energy for maintenance; NEg_{3x}: net energy for gain; NEL_{p3x}: net energy for lactation at a productive level of intake three times the maintenance level; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 19. Correlation study between FTIR protein structures and the protein portions of the in situ rumen incubation of canola meals

	CP (%DM)		Kd (%/h)		Fr (%)		T0 (%)		Residue 0h (%)	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.09	0.742	-0.10	0.733	0.08	0.781	-0.06	0.826	-0.18	0.524
A1	0.17	0.550	-0.01	0.970	0.06	0.830	0.01	0.987	-0.15	0.585
A2	0.00	0.990	-0.19	0.508	0.14	0.612	-0.08	0.776	-0.21	0.443
Area ratio	0.30	0.277	0.26	0.344	-0.20	0.478	-0.01	0.987	-0.06	0.825
A1H	0.15	0.585	-0.01	0.980	0.10	0.723	0.01	0.968	-0.30	0.283
A2H	-0.16	0.567	-0.22	0.427	0.27	0.337	-0.03	0.90	-0.03	0.924
Height	0.36	0.182	0.19	0.491	-0.20	0.475	-0.18	0.512	-0.33	0.232
Alpha	0.08	0.786	-0.10	0.723	0.16	0.576	-0.01	0.981	-0.33	0.229
Beta	0.13	0.647	-0.05	0.870	-0.00	0.990	-0.15	0.596	-0.12	0.666
Ratio	-0.01	0.985	-0.11	0.703	0.17	0.545	0.35	0.202	-0.29	0.289
	S (%)		D (%)		U (%)		BCP (%)			
	r	P value	r	P value	r	P value	r	P value		
Peak area	0.18	0.524	-0.11	0.685	0.03	0.930	0.10	0.723		
A1	-0.08	0.752	0.01	0.752	0.01	0.960	0.01	0.980		
A2	0.21	0.443	-0.15	0.603	0.08	0.781	0.18	0.533		
Area ratio	0.06	0.825	-0.02	0.935	-0.15	0.598	-0.35	0.201		
A1H	0.30	0.283	-0.21	0.45	0.05	0.870	-0.02	0.950		
A2H	0.03	0.924	-0.03	0.919	0.25	0.375	0.28	0.307		
Height	0.33	0.232	-0.23	0.420	-0.21	0.451	-0.36	0.191		
Alpha	0.33	0.229	-0.25	0.372	0.11	0.704	0.10	0.723		
Beta	0.12	0.666	-0.07	0.800	-0.05	0.870	0.03	0.920		
Ratio	0.29	0.289	-0.26	0.357	0.17	0.549	0.15	0.597		

DM: dry matter; CP: crude protein; Fr: Fermentation rate; Residue 0h: CP residue at 0h of rumen incubation ; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: potentially degradable fraction; U: rumen undegradable fraction; BCP: Bypass CP; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 20. Correlation study between FTIR protein structures and the protein portions of the in situ rumen incubation of canola seeds

	CP (%DM)		Kd (%/h)		Fr (%)		Residue 0h (%)	
	r	P value	r	P value	r	P value	r	P value
Peak area	-0.29	0.290	-0.07	0.810	0.13	0.657	-0.52	0.046
A1	-0.38	0.168	0.05	0.860	0.23	0.413	-0.43	0.111
A2	-0.24	0.390	-0.28	0.321	-0.05	0.860	-0.65	0.008
Area ratio	-0.15	0.594	0.60	0.019	0.47	0.074	0.66	0.007
A1H	-0.32	0.250	-0.25	0.368	0.01	0.970	-0.60	0.018
A2H	-0.11	0.694	-0.39	0.153	-0.16	0.567	-0.58	0.023
Height	-0.20	0.475	0.42	0.118	0.37	0.177	0.21	0.451
Alpha	-0.18	0.528	-0.36	0.189	-0.12	0.675	-0.48	0.072
Beta	-0.30	0.276	-0.02	0.945	0.28	0.317	-0.54	0.039
Ratio	0.12	0.676	-0.76	0.001	-0.60	0.019	-0.33	0.237
	S (%)		D (%)		U (%)		BCP (%)	
	r	P value	r	P value	r	P value	r	P value
Peak area	0.52	0.046	-0.44	0.105	0.04	0.894	-0.03	0.920
A1	0.43	0.111	-0.44	0.101	0.5	0.589	-0.07	0.800
A2	0.65	0008	-0.45	0.092	-0.18	0.516	0.09	0.742
Area ratio	-0.66	0.007	0.15	0.603	0.61	0.017	-0.26	0.355
A1H	0.60	0.018	-0.39	0.152	-0.10	0.712	0.10	0.717
A2H	0.58	0.023	-0.31	0.262	-0.26	0.354	0.22	0.443
Height	-0.21	0.451	-0.01	0.970	0.40	0.145	-0.19	0.508
Alpha	0.48	0.072	-0.23	0.401	-0.20	0.474	0.21	0.435
Beta	0.54	0.039	-0.51	0.053	0.19	0.500	-0.06	0.835
Ratio	0.33	0.237	0.16	0.576	-0.65	0.009	0.52	0.046

DM: dry matter; CP: crude protein; Fr: Fermentation rate; Residue 0h: CP residue at 0h of rumen incubation ; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: potentially degradable fraction; U: rumen undegradable fraction; BCP: Bypass CP; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 21. Correlation study between FTIR protein structures and the protein portions of the *in vitro* study of canola meals

	dBDM		IDBDM (%DM)		IDBDM (g/Kg DM)		TDDM (%DM)		TDDM (g/Kg DM)		IDP (%RUP)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.03	0.919	0.01	0.970	0.10	0.970	-0.07	0.810	-0.07	0.810	0.34	0.211
A1	0.00	1.000	-0.03	0.910	-0.03	0.910	-0.04	0.889	-0.04	0.889	0.28	0.318
A2	0.10	0.732	0.10	0.713	0.10	0.713	-0.08	0.771	-0.08	0.771	0.52	0.049
Area ratio	-0.24	0.395	-0.25	0.361	-0.25	0.36	-0.01	0.985	-0.01	0.985	-0.45	0.090
A1H	0.07	0.815	-0.09	0.752	-0.09	0.752	-0.06	0.830	-0.06	0.830	0.29	0.302
A2H	0.16	0.566	0.19	0.503	0.19	0.503	-0.17	0.545	-0.17	0.545	0.63	0.012
Height	-0.23	0.412	-0.26	0.355	-0.26	0.355	0.15	0.603	0.15	0.603	-0.38	0.159
Alpha	-0.01	0.955	-0.02	0.940	-0.02	0.940	-0.10	0.718	-0.10	0.718	0.37	0.172
Beta	0.05	0.864	0.04	0.899	0.04	0.899	-0.01	0.960	-0.01	0.960	0.26	0.354
Ratio	-0.15	0.588	-0.11	0.689	-0.11	0.689	-0.31	0.265	-0.31	0.265	0.19	0.490

	IADP (g/Kg DM)		IADP (g/Kg CP)		TDP (g/Kg DM)		TDP (g/Kg CP)		IADP (%CP)		TDP (%CP)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.27	0.328	0.15	0.603	0.14	0.621	0.24	0.390	0.15	0.603	0.24	0.390
A1	0.21	0.451	0.06	0.820	0.23	0.420	0.28	0.321	0.06	0.820	0.28	0.321
A2	0.39	0.148	0.26	0.348	0.13	0.657	0.36	0.182	0.26	0.348	0.36	0.182
Area ratio	-0.41	0.130	-0.44	0.100	0.16	0.567	-0.23	0.416	-0.44	0.100	-0.23	0.416
A1H	0.19	0.508	0.04	0.880	0.26	0.348	0.36	0.191	0.04	0.880	0.36	0.191
A2H	0.50	0.059	0.40	0.135	0.03	0.929	0.42	0.122	0.40	0.135	0.42	0.122
Height	-0.40	0.144	-0.47	0.089	0.27	0.328	-0.12	0.666	-0.47	0.079	-0.12	0.666
Alpha	0.26	0348	0.14	0.629	0.20	0.483	0.35	0.206	0.14	0.629	0.35	0.206
Beta	0.23	0.413	0.08	0.771	0.18	0.533	0.23	0.420	0.08	0.771	0.23	0.420
Ratio	0.19	0.503	0.12	0.666	0.12	0.671	0.23	0.412	0.12	0.666	0.23	0.412

DM: dry matter; CP: crude protein; RUP: Rumen undegradable protein; dBDM: Digestibility of bypass DM; IDBDM: Intestinally digestible rumen bypass DM; TDDM: Total digestible DM; dIDP: Intestinal digestibility of protein; IADP: Intestinally absorbable feed protein; TDP: Total digestible protein; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 22. Correlation study between FTIR protein structures and the protein portions of the *in vitro* study of canola seeds

	dBDM		IDBDM (%DM)		IDBDM (g/Kg DM)		TDDM (%DM)		TDDM (g/Kg DM)		IDP (%RUP)	
	r	P value	r	P value	r	P value	R0.09	P value	r	P value	r	P value
Peak area	-0.02	0.940	-0.16	0.567	-0.16	0.567	0.16	0.575	0.12	0.676	-0.20	0.483
A1	-0.04	0.880	-0.12	0.666	-0.12	0.666	0.09	0.761	0.04	0.880	-0.25	0.376
A2	0.05	0.860	-0.08	0.771	-0.08	0.771	0.31	0.258	0.30	0.283	-0.04	0.899
Area ratio	-0.13	0.657	0.05	0.860	0.05	0.860	-0.46	0.085	-0.49	0.064	-0.10	0.713
A1H	0.09	0.761	-0.06	0.820	-0.06	0.820	0.20	0.484	0.16	0.557	-0.11	0.689
A2H	0.19	0.495	-0.01	0.975	-0.01	0.975	0.45	0.094	0.43	0.114	0.15	0.593
Height	-0.38	0.164	-0.14	0.621	-0.14	0.621	-0.72	0.003	-0.74	0.002	-0.49	0.062
Alpha	0.28	0.321	0.11	0.690	0.11	0.690	0.23	0.418	0.19	0.491	0.11	0.704
Beta	-0.16	0.557	-0.32	0.245	-0.32	0.245	0.04	0.889	0.00	0.990	-0.27	0.327
Ratio	0.67	0.007	0.45	0.092	0.45	0.092	0.44	0.097	0.45	0.090	0.39	0.151
	IADP (g/Kg DM)		IADP (g/Kg CP)		TDP (g/Kg DM)		TDP (g/Kg CP)		IADP (%CP)		TDP (%CP)	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.05	0.860	-0.01	0.970	-0.23	0.413	0.05	0.860	0.01	0.970	0.04	0.889
A1	-0.13	0.657	-0.08	0.771	-0.20	0.483	0.13	0.648	-0.06	0.825	0.12	0.675
A2	0.11	0.685	0.13	0.648	-0.26	0.341	-0.07	0.810	0.14	0.616	-0.08	0.766
Area ratio	-0.35	0.206	-0.31	0.254	0.38	0.160	0.49	0.066	-0.31	0.256	0.50	0.056
A1H	008	0.770	0.11	0.689	-0.36	0.188	-0.10	0.727	0.13	0.644	-0.10	0.710
A2H	0.25	0.368	0.27	0.327	-0.22	0.427	-0.09	0.761	0.29	0.301	-0.10	0.732
Height	-0.40	0.144	-0.37	0.173	-0.01	0.970	0.09	0.742	-0.37	0.175	0.11	0.704
Alpha	0.25	0.368	0.28	0.308	-0.26	0.348	-0.06	0.830	0.30	0.276	-0.06	0.839
Beta	-0.17	0.536	-0.13	0.643	-0.20	0.470	0.03	0.919	-0.11	0.694	0.02	0.932
Ratio	0.65	0.009	0.34	0.011	-0.39	0.148	-0.46	0.081	0.65	0.009	-0.46	0.085

DM: dry matter; CP: crude protein; RUP: Rumen undegradable protein; dBDM: Digestibility of bypass DM; IDBDM: Intestinally digestible rumen bypass DM; TDDM: Total digestible DM; dIDP: Intestinal digestibility of protein; IADP: Intestinally absorbable feed protein; TDP: Total digestible protein; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet.
r: correlation coefficient using Spearman.

A. 23. Correlation study between FTIR protein structures of canola meals and the NRC model

	MCP_RDP		MCP_TDN		AMCP		RUP		ARUP	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.07	0.800	-0.01	0.970	-0.03	0.924	0.21	0.459	0.26	0.355
A1	0.02	0.940	-0.01	0.960	-0.03	0.909	0.13	0.657	0.18	0.524
A2	-0.15	0.585	0.10	0.733	0.08	0.771	0.23	0.413	0.36	0.191
Area	0.37	0.172	-0.09	0.756	-0.08	0.776	-0.22	0.439	-0.41	0.134
A1H	0.04	0.880	0.05	0.860	0.03	0.914	0.07	0.800	0.14	0.612
A2H	-0.29	0.301	0.30	0.271	0.29	0.291	0.33	0.226	0.50	0.056
Height	0.39	0.148	-0.24	0.383	-0.24	0.394	-0.31	0.266	-0.44	0.101
Alpha	-0.06	0.830	0.15	0.584	0.14	0.622	0.16	0.554	0.28	0.372
Beta	-0.01	0.970	0.05	0.870	0.03	0.924	0.15	0.603	0.17	0.541
Ratio	-0.05	0.864	0.14	0.615	0.14	0.624	0.15	0.593	0.20	0.478
	AECP		MP		DPB		NRC FMV		ECP	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.08	0.779	0.27	0.334	-0.09	0.761	0.27	0.334	0.06	0.835
A1	0.06	0.839	0.19	0.499	0.00	0.990	0.19	0.499	0.04	0.884
A2	0.15	0.596	0.39	0.156	-0.18	0.516	0.39	0.156	0.13	0.643
Area	0.03	0.924	-0.38	0.166	0.36	0.184	-0.38	0.166	0.02	0.939
A1H	0.13	0.655	0.16	0.559	0.03	0.910	0.16	0.559	0.11	0.694
A2H	0.24	0.386	0.53	0.043	-0.33	0.228	0.53	0.043	0.20	0.467
Height	-0.11	0.721	-0.41	0.132	0.40	0.140	-0.41	0.132	-0.08	0.780
Alpha	0.15	0.595	0.27	0.328	-0.06	0.820	0.27	0.327	0.12	0.659
Beta	-0.02	0.954	0.19	0.499	-0.04	0.889	0.19	0.499	-0.03	0.909
Ratio	0.06	0.826	0.18	0.528	0.00	0.995	0.18	0.528	0.04	0.884

MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN_{3x}; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; NRC FMV: estimated milk production based on the NRC system in kg milk/kg DM feed; ECP: endogenous protein in the small intestine; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 24. Correlation study between FTIR protein structures of canola seeds and the NRC model

	MCP_RDP		MCP_TDN		AMCP		RUP		ARUP	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	-0.02	0.940	-0.27	0.328	-0.27	0.328	-0.11	0.704	-0.15	0.594
A1	-0.06	0.840	-0.32	0.243	-0.32	0.243	-0.18	0.533	-0.21	0.451
A2	-0.04	0.880	-0.11	0.685	-0.11	0.685	0.03	0.930	-0.01	0.970
Area	-0.03	0.930	-0.46	0.084	-0.46	0.084	-0.30	0.277	-0.18	0.533
A1H	-0.15	0.593	-0.22	0.423	-0.22	0.423	0.03	0.909	-0.04	0.889
A2H	-0.06	0.845	-0.21	0.455	-0.21	0.455	0.16	0.558	0.17	0.545
Height	-0.13	0.648	0.10	0.733	0.10	0.733	-0.24	0.390	-0.44	0.105
Alpha	-0.23	0.412	-0.44	0.098	-0.44	0.098	0.15	0.589	0.16	0.580
Beta	0.03	0.904	-0.15	0.593	-0.15	0.593	-0.08	0.771	-0.20	0.478
Ratio	-0.44	0.101	-0.28	0.315	-0.28	0.315	0.49	0.066	0.48	0.069
	AECP		MP		DPB		NRC FMV		ECP	
	r	P value	r	P value	r	P value	r	P value	r	P value
Peak area	0.37	0.170	-0.40	0.144	0.35	0.198	-0.40	0.143	0.39	0.147
A1	0.27	0.339	-0.48	0.074	0.39	0.153	-0.48	0.072	0.28	0.307
A2	0.54	0.040	-0.16	0.558	0.21	0.462	-0.17	0.549	0.55	0.033
Area	-0.50	0.060	-0.41	0.125	0.37	0.177	-0.41	0.131	-0.51	0.055
A1H	0.48	0.072	-0.27	0.330	0.25	0.372	-0.26	0.344	0.49	0.063
A2H	0.60	0.019	-0.07	0.805	0.25	0.372	-0.07	0.810	0.61	0.016
Height	-0.45	0.089	-0.26	0.341	-0.20	0.474	-0.26	0.354	-0.45	0.089
Alpha	0.63	0.013	-0.24	0.390	0.36	0.188	-0.23	0.417	0.63	0.012
Beta	0.17	0.556	-0.35	0.203	0.28	0.317	-0.33	0.226	0.20	0.483
Ratio	0.88	<0.001	0.14	0.630	0.02	0.955	0.15	0.597	0.84	<0.001

MCP_RDP: microbial protein synthesized in the rumen based on available RDP (rumen degradable protein); MCP_TDN: microbial protein synthesized in the rumen based on TDN_{3x}; AMCP: rumen synthesized microbial protein truly absorbed in the small intestine; RUP: rumen undegradable protein; ARUP: RUP truly absorbed in the small intestine; AECP: truly absorbed ECP in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; NRC FMV: estimated milk production based on the NRC system in kg milk/kg DM feed; ECP: endogenous protein in the small intestine; Peak area: Amide I and II peak area; AI: Amide I area; AII: Amide II area; Area ratio: ratios of amide I and amide II areas; A1H: Amide I height; A2H: Amide II height; Height: ratios of amide I and II heights; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet. r: correlation coefficient using Spearman.

A. 25. Regression analysis to predict canola protein parameters from FTIR protein structures

Variables (Y)	Prediction equation model: Y=a + b1 x X1 + b2 x X2 …	R^2	RSD	*p* value
	Canola meals			
CP (%DM)	Y = 38.11 – 15.61 x A2H + 20.90 x Alpha	0.23	0.89	0.004
TD (%CP)	Y = 37.09 – 17.80 x A2H + 22.43 x Alpha	0.24	0.90	0.004
TDN_{1x}	Y = 62.78 + 13.64 x A2H	0.23	1.38	<0.001
DE_{1x}	Y = 3.09 + 0.66 x Alpha	0.27	0.06	<0.001
DEp_{3x}	Y = 3.03 + 0.41 x Alpha	0.27	0.04	<0.001
ME_{3x}	Y = 2.54 + 0.54 x Alpha	0.27	0.05	<0.001
MEp_{3x}	Y = 2.61 + 0.41 x Alpha	0.27	0.04	<0.001
Nem_{3x}	Y = 1.66 + 0.45 x Alpha	0.25	0.04	<0.001
NEg_{3x}	Y = 1.03 +0.40 x Alpha	0.26	0.04	<0.001
NEL_{p3x}	Y = 1.65 + 0.29 x Alpha	0.25	0.03	<0.001
Estimated Milk Value (FMV)	Y = 2.46 + 0.44 x Alpha	0.26	0.04	<0.001
	Canola seeds			
CP (%DM)	Y = 24.79 + 0.79 x Area – 2.19 x Height	0.30	0.46	<0.001
TD (%CP)	Y = 24.51 + 0.85 x Area – 2.34 x Height	0.33	0.47	<0.001
S (%DM)	Y = 81.96 – 20.51 x Area	0.33	3.42	0.025
D (%DM)	Y = 84.32 – 86.95 x Beta	0.32	2.77	0.029
U (%DM)	Y = - 31.42 + 17.41 x Area	0.28	3.29	0.045
IADP (%CP)	Y = - 254.82 + 307.25 x Ratio	0.35	24.18	0.020
PA2 (%CP)	Y = 33.36 + 5.24 x A1 – 367.58 x Ratio	0.72	3.36	<0.001
PB1 (%TP)	Y = 75.75 – 123.04 x Alpha	0.43	4.63	0.008
RDPA2 (%DM)	Y = 5.88 + 0.83 x A1 – 59.31 x Alpha	0.68	0.56	0.001
RDPB1 (%DM)	Y = 4.44 – 0.47 x A1 + 34.84 x Beta	0.64	0.33	0.002
Total RDP (%DM)	Y = 9.73 + 8.56 x Alpha	0.42	0.33	0.009
RUPA2 (%DM)	Y = 2.35 + 0.33 x A1 -23.72 x Beta	0.68	0.23	0.001
RUPB1(%DM)	Y = 6.70 + 0.70 x A1 + 52.15 x Beta	0.65	0.50	0.002
Total RUP (%DM)	Y = 12.59 – 0.40 x A1 + 25.35 x Beta	0.75	0.25	<0.001
DVE (%DM)	Y = - 27.01 + 77.08 x Ratio	0.32	6.51	0.029
OEB (%DM)	Y = 200.10 – 81.49 x Ratio	0.36	6.32	0.019
DVE (FMV)	Y = - 0.55 + 1.56 x Ratio	0.32	0.13	0.028
AECP (%DM)	Y = 3.96 – 0.15 x Area + 0.76 x Ratio	0.82	0.03	<0.001
ECP (%DM)	Y = 9.97 – 0.41 x Area + 1.90 x Ratio	0.81	0.07	<0.001

RSD: residual standard deviation; Alpha: α-helix height; Beta: β-sheet height; Ratio: ratio of α-helix: β-sheet; A2H: Amide II height; AI: Amide I area; CP: crude protein; TDCP: Total digestible CP; TDN_{1x}: total digestible nutrients at one time maintenance level; $DE_{1x:}$: digestible energy at a one time maintenance level; DE_{p3x}: digestible energy at a productive level of intake three times the maintenance level; ME_{3x}: metabolizable energy for gain at three times the maintenance level; ME_{p3x}: metabolizable energy at a productive level of intake three times the maintenance level; NEm_{3x}: net energy for maintenance; NEg_{3x}: net energy for gain; NEL_{p3x}: net energy for lactation at a productive level of intake three times the maintenance level; S: soluble fraction; D: potentially degradable fraction; U: rumen undegradable fraction; PA2: soluble true protein; PB1: moderately degradable protein; Total RDP: total rumen degradable protein; Total RUP: total rumen undegradable protein; DVE: truly digested protein in the small intestine; OEB: degradable protein balance; DVE FMV: estimated milk production based on the DVE system in kg milk/kg DM feed; AECP: truly absorbed ECP in the small intestine; ECP: endogenous protein in the small intestine.