Quantify Protein Structure and Subfractions in Canola Seed and Canola Meal in Relation to Nutrient Availability in Animals

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Final Report

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Executive Summary

This final report provides information on the current status of this canola research program to quantify protein structure and subfractions in canola seed and canola meal in relation to nutrient availability in animals. The objectives of this research program were: Short-term: To determine the protein quality of both canola seeds and canola meal in terms of protein subfractions and total truly absorbed protein value in ruminants (In this way we know whether the industry processing affects protein value and subfractions in canola meal, compared to original canola seed); To detect protein molecular structure features of both canola seeds and canola meal using advanced synchrotron-radiation based infrared microspectroscopy (SR-IMS) and globar sourced FTIR molecular spectroscopy (In this way we know whether the industry processing change protein molecular structure in canola meal, compared to original canola seed); To study the effects of heat processing of canola at various conditions (different times and temperatures) on protein molecular structure changes, protein subfractions and total truly absorbed protein value; To detect the sensitivity of canola protein intrinsic structure to the industry processing (physical and chemical) and heat-processing and to study processing-induced changes of protein molecular structure on nutrient availability in animals; To quantify protein structure changes in relation to protein utilization and availability in animals; To provide an insight why an equivocal performance effect exists when feeding canola meal as a protein source to animals (eg. dairy cattle) though newly developed modeling approach; To train highly qualified personal (HQP: PhD student or PDF) for Saskatchewan Agriculture as one of high priority in this proposed research program. Long-term: To advance our current protein structure and protein nutrition knowledge by increasing and enhancing basic knowledge of the nutritional relevance of canola protein intrinsic structure and chemistry in a molecular basis.

Based on the above objective, we designed the following three programs with eight main projects to answer the above questions.

- Program I: Research Program in Effect of Processing Conditions, Different Types of Co-Products (Canola Meal and Presscake) and Yellow and Brown Meal: Comparison of Yellow and Brown-Seeded Canola Meal with the Brown-Seeded Canola Presscake (completed).
- Project 1: EFFECT OF PROCESSING CONDITIONS ON THE NUTRITIVE VALUE OF CANOLA MEAL AND PRESSCAKE: COMPARISON OF THE YELLOW AND BROWN-SEEDED CANOLA MEAL WITH THE BROWN-SEEDED CANOLA PRESSCAKE (COMPLETED)
- Project 2: METABOLIC CHARACTERISTICS OF THE PROTEINS IN YELLOW- SEEDED AND BROWN-SEEDED CANOLA MEAL AND PRESSCAKE IN DAIRY CATTLE: COMPARISON OF THREE SYSTEMS (PDI, DVE, AND NRC) IN NUTRIENT SUPPLY AND FEED MILK VALUE (FMV) (COMPLETED)
- Project 3: REVELATION OF PROTEIN MOLECULAR STRUCTURES OF CANOLA MEAL AND PRESSCAKE, AS AFFECTED BY HEAT-PROCESSING METHODS, IN RELATIONSHIP WITH THEIR PROTEIN DIGESTIVE BEHAVIOR AND UTILIZATION FOR DAIRY CATTLE (COMPLETED)
- Program II: Research Program in Newly Developed Yellow-Seeded and Black-Seeded Canola Lines (completed).
- Project 4: MAGNITUDE DIFFERENCES IN BIOACTIVE COMPOUNDS, CHEMICAL FUNCTIONAL GROUPS, FATTY ACID PROFILES, NUTRIENT DEGRADATION AND DIGESTION, MOLECULAR STRUCTURE, AND METABOLIC CHARACTERISTICS OF PROTEIN IN NEWLY DEVELOPED YELLOW-SEEDED AND BLACK-SEEDED CANOLA LINES (COMPLETED)
- Project 5: PROTEIN AND ENERGY METABOLIC CHARACTERISTICS AND NUTRIENT SUPPLY TO RUMINANTS FROM NEWLY DEVELOPED CANOLA LINES, AS PREDICTED USING THE NRC-2001 MODEL AND THE PDI SYSTEM (COMPLETED) *
- Project 6: EXPLORE PROTEIN MOLECULAR STRUCTURE IN ENDOSPERM TISSUES IN

NEWLY DEVELOPED BLACK AND YELLOW TYPE CANOLA SEEDS BY USING SYNCHROTRON-BASED FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY (COMPLETED)

- Program III: Graduate Reseach Thesis Projects.
- Project 7: 1st MSC THESIS PROJECT: IMPROVEMENTS IN NUTRITIVE VALUE OF CANOLA MEAL WITH PELLETING (COMPLETED) (COMPLETED) *
- Project 8: 2nd MSC THESIS PROJECT: STRUCTURAL, PHYSIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF NEWLY DEVELOPED CANOLA SEEDS and BRASSICA CARINATA AND THE CO-PRODUCTS.

This research was carried out in collaborations with the various scientists from different research institutes including Agriculture and Agri-Food Canada (AAFC: Sally Vail, Kevin Falk), Canadian Feed Research Center (CFRC), Feed Innovation Institute (FII), Brookhaven National Lab, Canadian Feed Research Center (CFRC), and Dept of Animal and Poultry Science Uof S.

Highly Qualified Personnal (HPQ) Training included three graduate students and postdoctoral fellow: Mr. Xuewei. Huang and Ms. Yajing Ban were hired and brought into the project as MSc students, Ms. Xinxin Li was brough into project as a joint taining PhD student under the CSC scholarship. Katerina Theodoridou was hired and brought into the project as PDF fellows at different stages. Dr Yu's other team members: H. Xin (PDF fellow, University of Saskatchewan) and Zhiyuan Niu (Research Assistant, University of Saskatchewan) were also involved in the program and carried out part of studies and/or provided various research assistance to this program.

Interesting research findings from this program have been published in various peer-reviewed scientific journals with high impact factors, such as the Journal of Dairy Science (USA), the Journal of Agriculture and Food Chemistry (USA) , the Molecular and Biomolecular Spectroscopy (see following publication and presentation list section for details).

In order to make our research results available to farmers, producters, nutritionists and researchers as part of our tech transfer/extention acitiviteis, we have talked our research and presented our work and interesting findings at various industry meetings and professional meetings either as invtieted speaker or as contributors, such as The Western Canadain Dairy Seminar, the Western Nutrition Conference - Processing, Performance & Profit; The Annual Dairy Info Day; The CSAS-ADSA-ASAS Joint Annual Meeting (see following talk and presentation list section for details).

In order to let more farmers, producers and industry people know our research findings, we have also written extension articles in a plain language and published our findings in Industry journals/Magazine, Newsletter and Press (see following presentation and industry article list section for details).

The research program has been attacted by the CTV-News-Farm Gate and The Western Producers. They interviewed my project team members and introduce the findings.

Research Progress

Introduction: Research Motivation and Background

Canola, including the yellow-seeded Brassica rapa and dark-brown seeded Brassica napus varieties, is grown in Western Canada. Normally we use "wet" chemical analysis to analyze nutritive value of canola seed and canola meal. However, conventional "wet" chemical analysis fails to reveal canola protein structure and structural matrix information (Yu, 2004) and fails to link structural information to chemical information (Budevska 2002). An understanding of relationships between feed protein (in terms of protein content, intrinsic structure, and protein matrix) and nutrient utilization and availability in animal are important to economical and sustainable animal productions. Protein quality, characteristics, utilization and digestive behaviour are closely related to: not only 1) total protein composition (such as crude protein content or amino acid profile), but also 2) protein intrinsic structures (such as protein conformation (ratio and % of α-helix, β-sheet, random coil, and beta turns), protein structural change during processing eg heating), and 3) protein matrix in canola seed and meal. The problem is that

conventional "wet" chemical analysis that we are using can only determine total protein composition or amino acid composition, but fails to detect the protein intrinsic structures and component matrix and fails to link structural information to chemical information (Budevska, 2002; Yu, 2004). This is one of reasons why an equivocal performance effect exists when feeding canola meal as protein source to dairy cattle. In NRC-2001 Dairy and NRC-1996 Beef, both suggest use a chemical summary approach (without considering feed intrinsic structures) to determine feed value such as TDN, DE, ME and NE values. This NRC approach fails to accurately determine feed quality. That is why an equivocal performance effect exists when feeding canola meal as protein source to dairy cattle.

Studying the structure of proteins leads to an understanding of the components that make up a whole protein (Dyson and Wright 1990; Carey 1996). An understanding of the structure of the whole protein is often vital to understanding its digestive behaviour, nutritive quality, utilization and availability in animals. Protein structures include main α-helix and β-sheet, and small amount of β-turn and random coil (Carey 1996). The percentage of these structures influences protein quality, nutrient utilization, availability and digestive behaviour. The protein structural profiles affect access to gastrointestinal digestive enzymes, which affects protein value and protein availability. However, studies on protein structures in relation to nutritive value and digestive behaviours of protein in animals are rarely. Our primary research has shown that different protein sources have different percentages and ratios of α-helix and β-sheet in protein structures. These protein structures were also highly associates with protein nutritive value.

Heat processing impact feed protein utilization and availability in animals. Sometime it reduces the amounts of protein entering the small intestine for absorption and digestion (Yu et al. 2002). The basic mechanism of altering the protein digestive behaviour (Goelema 1999) with heat processing involves denaturation, unfolding or uncoiling of a coiled or pleated structure (Holum 1982). Any temperature change in the environment of the protein which can influence the non-covalent interactions involved in the structure may lead to an alteration of the protein structure (Goelema 1999) including protein secondary structures. New research (Seguchi et al. 2004) show that heat processing affected protein secondary structures and change the α-helix to β-sheet ratio. These changes affected nutritive quality of egg protein. Canola processing includes various physical, chemical and heat processing. We still don't know the processing-induced changes of canola protein structure. We still don't know how sensitive of canola protein molecular structure to the various physical, chemical and heat processing.

Published review shows that the effects of heat processing on protein nutritive value, utilization and availability and performance in animals are very equivocal. Part of reason is that heating conditions of inside a feed is not optimal, the feed being either underheated or overheated. To assess the protein digestive behaviour in ruminants, the Cornell net carbohydrate protein system (Sniffen el al., 1992; Chalupa and Sniffen, 1994) is, one of methods, often used to partition the protein fractions in five subfractions to relate to degradation behaviours in the rumen. One novel approach proposed here to check the effects of the processing on canola protein value and nutrient availability is to look at magnitude of changes of protein intrinsic molecular structures in canola seed affected by various processing. The newly developed and cutting-edge SR-IMS technique enables us to locate relatively "pure" protein body/tissues in the canola seed. With this advanced technique, we can quantify relative percentages of α-helix and β-sheet as well as α-helix to β-sheet ratio within the intact tissue (Wetzel et al., 2003; Yu, 2008, 2010). Most animal studies on feeding canola have only focused on total protein or AA composition affected by heat processing using traditional "wet" chemical analysis without consideration of any canola inherent structural and matrix effects (Yu, 2004). As we know, the protein value, quality, utilization and availability and digestive behaviour are closely related to not only total protein or AA composition, but also inherent structures and component matrix.

Advanced synchrotron radiation based infrared microspectroscopy (SR-IMS) has been recently as a rapid, direct, non-invasive, non-destructive and bio-analytical technique. It takes advantages of synchrotron light brightness (million times brighter than sunlight) and small effective source size, is capable of exploring the molecular chemistry within structures of biological tissues without destruction inherent structures within cellular dimension (Wetzel et al. 1998; Wetzel 2001; Marinkovic et al. 2002; Miller 2000, 2002; Yu, 2008). With the synchrotron technology, localization of "pure" protein and protein structural characteristics is achievable. The protein structural features could be revealed at cellular and

molecular levels (Wetzel et al. 2003; Yu et al., 2007).

To date, no systematic study has been carried out to study the ultra-structural chemical and nutritive features of protein structures in canola seed affected by various processing using advanced synchrotron technology in relation to protein quality, utilization and availability in animals. This research aims to use the advanced synchrotron technology to reveal molecular chemistry of protein structures of canola seeds affected by the processing within intact tissue within cellular dimensions, to detect the sensitivity of canola protein intrinsic structure to industry processing and processing-induced changes on nutrient availability in animals and quantify protein secondary structures using multi-component peak modeling Gaussian and Lorentzian methods (Yu 2005), in relation to protein digestive behaviours and nutritive value in the rumen. Information from the study by probing canola protein structures will be valuable as a guide to maintain protein quality and to provide an insight why an equivocal performance effect exist when feeding canola meal as protein source to animals (eg. dairy cattle) though modeling nutrient supply. It is believed that by using the advanced synchrotron technology, it will make a significant step and an important contribution to canola protein nutritional research. It is anticipated that the proposed program will provide with a greater understanding the canola-animal interface and advance our current and basic canola protein nutrition knowledge, benefit canola industry and increase the competitive position of Saskatchewan canola and increase economic returns to Saskatchewan.

Overall Project Objectives:

Long-term:

To advance our current protein structure and protein nutrition knowledge by increasing and enhancing basic knowledge of the nutritional relevance of canola protein intrinsic structure and chemistry in a molecular basis.

Short-term:

- To determine the protein quality of both canola seeds and canola meal in terms of protein subfractions and total truly absorbed protein value in ruminants (In this way we will know whether the industry processing affects protein value and subfractions in canola meal, compared to original canola seed)
- To detect protein molecular structure features of both canola seeds and canola meal using advanced synchrotron-radiation based infrared microspectroscopy (SR-IMS) and Globar molecular spectroscopy. (In this way we will know whether the industry processing change protein molecular structure in canola meal, compared to original canola seed)
- To study the effects of heat processing of canola at various conditions (different times and temperatures) on protein molecular structure changes, protein subfractions and total truly absorbed protein value.
- To detect the sensitivity of canola protein intrinsic structure to the industry processing and heat-processing and to study processing-induced changes of protein molecular structure on nutrient availability in animals.
- To quantify protein structure changes in relation to protein utilization and availability in animals.
- To train highly qualified personal (HQP: PhD student or PDF) for Saskatchewan Agriculture as one of high priority in this proposed research program.

Program I:

Research Program in Effect of Processing Conditions, Different Types of Co-Products (Canola Meal and Presscake) and Yellow and Brown Meal: Comparison of Yellow and Brown-Seeded Canola Meal with the Brown-Seeded Canola Presscake

The Program I includes the following three main projects.

Project 1: EFFECT OF PROCESSING CONDITIONS ON THE NUTRITIONAL VALUE OF CANOLA MEAL AND CANOLA PRESSCAKE FOR RUMINANTS: COMPARISON OF YELLOW-SEEDED (BRASSICA JUNCEA) AND BROWN-SEEDED (BRASSICA NAPUS) CANOLA MEAL WITH BROWN-SEEDED (BRASSICA NAPUS) CANOLA PRESSCAKE (COMPLETED) *

A version of this project has been published: Katerina Theodoridou, Peiqiang Yu. 2013. Effect of Processing Conditions on the Nutritive Value of Canola Meal and Canola Presscake: Comparison of the Yellow-Seeded (Brassica Juncea) and the Brown-Seeded (Brassica Napus) Canola Meal with the Brown-Seeded (Brassica Napus) Canola Presscake. Journal of Science of Food and Agriculture (England). 93:1986-95 (DOI: 10.1002/jsfa.6004) (*as supervisor, PI, corresponding author).

Project Details

Abstract

BACKGROUND: Canola, unlike with traditional rapeseed, contains low levels of "erucic acid" and "glucosinolates. Canola's industrial processing, generally involves separation of the seed into an oil and a meal fraction whereas the intermediate product called presscake. The objective of this study was to determine the effect of processing conditions on the nutritive value, for ruminants and compare the yellow (*B. juncea*) (CM_Y), brown-seeded (*B. napus*) (CM_B) canola meal and the brown-seeded (*B. napus)* (CPC_B) canola presscake.

RESULTS: Either extract was higher (P<0.05) for CPC_B than for CM_Y and CM_B. CM_Y had a higher (P<0.05) PB2 and PC compared to CM_B. Truly digestible fractions, except fatty acid, were lower (P<0.05) for CPC_B. Effective protein degradability of CM_Y was lower (P<0.05) compare to CM_B or CPC_B. *In vitro* protein intestinal digestibility for CM_Y was higher (P<0.05) than for CM_B or CPC_B.

CONCLUSION: CPC_B had lower protein but higher oil content than canola meal and is a potential great energy supplement source for ruminants. CM_Y had higher crude protein, digestible and metabolizable energy values and low fiber than CM_B. In plant breeding, selection programmes are geared towards yellow-seeded varieties in order to improve the nutritive value of canola meal.

Keywords: Canola, Processing method, Protein evaluation, In *situ* measurements, Intestinal digestibility,

Introduction

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in 1970's. Unlike with traditional rapeseed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30 μmol of any one or any combination of the four aliphatic glucosinolates in its defatted meal.[1] Its industrial processing generally involves separation of the seed into an oil and a meal fraction. Canola meal contains a relatively high amount of fiber due to the high content (30 %) of hull in the meal. Attempts have been made to improve the nutritional value of the meal by increasing the digestibility of the hull and/or reducing the hull proportion in the meal. Canola meal includes the yellow-seeded and brown-seeded varieties; whereas hulls from yellow-seeded have been reported to be lower in fiber than those from brown-seeded types.[2-5] Plant selection programs have been directed toward increased yellow seed content to decrease the fiber content. In addition to containing less fibre, yellow-seeded varieties of canola have been shown to be higher in oil and protein content than brown-seeded varieties.[2,5]

The intermediate product in the manufacturing process of canola oil and canola meal called canola presscake. Canola presscake is partially oil extracted canola seed that has been flaked, cooked and expeller-processed but not solvent-extracted or exposed to the high temperature of the desolventizer-toaster as is the case for commercial canola meal.[6] However, since presscake has not passed through the solvent extraction process, the oil content is higher while crude protein is lower than in canola meal.[6,7] The different kind of heat treatments applied in animal feed processing are able to improve nutrient utilization and availability of canola. The heat processing can reduce the solubility of nutrients, decreasing rumen fermentation and degradability, while increasing available by passing the rumen for intestinal digestion and absorption. Several researchers have shown that heat treatment reduces protein solubility and increases ruminal undegraded protein of canola seed[8], canola presscake[9] and canola meal.[10]

Objective

In the present study, we hypothesized that the nutritional value and nutrient degradation characteristics depending on processing conditions and that canola meal differs from canola presscake. The objective of this research work was to characterize the effect of processing conditions on the nutritional value, for ruminants, of the yellow-seeded (*B. juncea*) and brown-seeded (*B. napus*) canila meal, in comparison with brown-seeded (*B. napus)* canola presscake. The items assessed included (1) chemical and nutrient profiles, (2) energy value, 3) protein and carbohydrate subfractions, (4) rumen degradation kinetics and 5) *in vitro* intestinal feed protein digestibility.

Material and Methods

The animals used in this study were cared for according to the guidelines put forward by the Canadian Council on Animal Care (1993).[11]

Feedstuffs

Canola meal (CM) and canola presscake (CPC) were used in this study as a feed protein source. A 4-kg sample of yellow-seeded (CM_Y) (*B. juncea)* and brown-seeded (CM_B) (*B. napus*) solvent-extracted canola meal were obtained from the feed mill of University of Saskatchewan. A 10-kg sample of brown-seeded (*B. napus)* canola presscake (CPC_B) was produced and obtained from Milligan Biotech (Foam Lake, Saskatchewan).

Animals and Diets

Three dry Holstein cows fitted with a rumen cannula with an internal diameter of 10 cm (Bar Diamond, Parma, ID) were used for the *in situ* rumen degradation parameters. Cows were housed in the research barn at the University of Saskatchewan during the period of study. The cows were given *ad*

libitum access to water and individually fed 15 kg (as fed) of a totally mixed ration (TMR) twice daily (7.5 kg/feeding) at 0800 and 1600 formulated to meet or exceed NRC Nutrient Requirements (2001). The total mixed ration consisted of 57% barley silage, 10% alfalfa hay, 5% dehydrated alfalfa pellets and 28% concentrates (containing barley, wheat, oats, canola meal, soybean meal, wheat DDGS, corn gluten meal, molasses, golden flakes, canola oil, minerals and vitamins).

Rumen Incubation Procedure

Seven grams of an individual ground sample were weighed into a pre-weighed and numbered nylon bag (10 x 20 cm; Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississauga, ON) with the pore size of approximately 40 μm. These bags were tied about 2 cm below the top, allowing a ratio of sample size to bag surface area of 19 mg/cm^2. Samples were incubated in the rumens for 0, 2, 4, 8, 12, 24, and 48 h. Rumen incubations were performed according to the 'gradual addition/all out' schedule.[12] In this technique, the bags assigned for the longest incubation time (48 h), are put in the rumen first and then after 24 h since the first bags have been incubated in the rumen, the next bags with the next longest incubation time (24 h) are added and so on. The maximum number of bags in the rumen at any one time was 30.[12] After the incubation the bags were removed from the rumen and rinsed under a cold stream of tap water to remove excess ruminal contents. The bags were washed with cool water without detergent and subsequently dried at 55°C for 48 h and reweighed to complete the calculation. The 0 h incubation samples were only washed under the same conditions. The dried samples were kept in a refrigerated room (4°C) until needed for chemical analysis.

Chemical Analysis

Laboratory samples of canola meal, canola presscake and rumen residues collected from the nylon bags, were pooled and transferred into labeled containers, were prepared by grinding to pass a 1 mm screen (Retsch ZM-1; Brinkmann Instruments, Mississauga, ON) and analysed for dry matter (DM) (AOAC official method 930.15), ash (AOAC official method 942.05), ether extract (EE), (AOAC official method 954.02) and crude protein (CP) (Leco Protein/NAnalyser FP-528, Leco Corp., St Joseph, MI, USA) contents according to AOAC (1990).[13] Acid detergent lignin (ADL) and neutral detergent fiber (NDF) were determined according to Van Soest *et al.*[14] with sodium sulfite and heat stable alpha amylase and expressed including residual ash. Acid detergent fiber (ADF) was determined according to AOAC and expressed with the inclusion of residual ash. The NDF, ADL and ADF procedures were adapted for use in an Ankom 200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). In order to prevent the high fat content of canola samples, from giving inaccurately high values for NDF, fat was extracted by incubating the samples in acetone for 2 h. All samples were analysed in duplicate; the analysis was repeated if the error was higher than 5%. Acid detergent insoluble nitrogen (ADIN)[15], neutral detergent insoluble nitrogen (NDIN)[15], non-protein nitrogen (NPN)[16], acid detergent insoluble protein (ADIP) and neutral detergent insoluble protein (NDIP) were calculated as ADIP = 6.25 × ADIN and NDIP = 6.25 × NDIN, respectively. The non-protein N (NPN) content was analyzed by precipitating of true protein with tungstic acid (samples were soaked into water with 0.3 mol L^{-1} Na_2WO_4 for 30 min) and calculated as the difference between total N and the N content of the residue after filtration. Soluble CP (SCP) was determined by incubating the sample with bicarbonate-phosphate buffer and filtering through Whatman #54 filter paper.[16] Neutral detergent fiber was analyzed with the addition of sodium sulfite and with the inclusion of heat stable α-amylase.[14] Total carbohydrate (CHO) was calculated as: CHO = 100 – EE – CP – ash (NRC, 2001).[17] Non-fiber carbohydrate was calculated as: NFC = 100 – (NDF – NDIP) – EE – CP – ash (NRC, 2001).[17] Condensed tannins (CT) concentrations were measured using HCl-butanol.[18]

Energy Values

Estimated energy contents for total digestible CP (tdCP), fatty acid (tdFA), NDF (tdNDF) and non-fiber carbohydrate tdNFC) and total digestible nutrient at 1×maintenance (TDN_{1X}), digestible energy at production level of intake (DE_{3x}), metabolisable energy at production level of intake (ME_{3x}) and net energy for lactation at production level of intake (NEL_{3x}) were predicted using a summative approach

from the NRC 2001 dairy[17], while net energy for maintenance (NE_m), and net energy for growth (NE_g) were predicted using the NRC 1996 beef.[19] Both NRC dairy and NRC beef used the same formula to estimate NE_g and NE_m.

Fractionating Protein Fractions

Crude protein fractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS).[20] The characterizations of the CP fractions used in this system are as follows: fraction PA is non-protein nitrogen (NPN), fraction PB is true protein, and fraction PC is unavailable protein. Fraction PB is further divided into 3 fractions (PB1, PB2, and PB3) that are believed to have different rates of degradation in the rumen. Fraction PB1 was calculated as PB – PB2 – PB3. Buffer-insoluble protein minus fraction PB3 is used to estimate fraction PB2. Fraction PB2 is insoluble in buffer but soluble in neutral detergent, whereas fraction PB3 is insoluble in both buffer and neutral detergent, but is soluble in acid detergent. Fraction PB2 is fermented in the rumen at a lower rate than buffer-soluble fractions, and some of the PB2 fraction escapes to the lower gut. Fraction PB3 is believed to be more slowly degraded in the rumen than fractions PB1 and PB2 because of its association with the plant cell wall; a large proportion of PB3 is thus believed to escape the rumen. Fraction PC is the acid detergent insoluble N, which is highly resistant to breakdown by microbial and mammalian enzymes, and it is assumed to be unavailable for the animal.[20] The relative rumen degradation rates of the five protein fractions have been described by Sniffen *et al.*[20] as follows: fractions PA is assumed to be infinity, fraction PB1 is 1.20–4.00 h^{-1}, fraction PB2 is 0.03–0.16 h^{-1}, fraction PB3 is 0.0006–0.0055 h^{-1}. Fraction PC is considered to be undegradable.

Fractionating Carbohydrate Fractions

Carbohydrate was partitioned into: a rapidly degradable fraction (CA) which is composed of fermentable soluble sugars that have a rapid degradation rate of 3.00 h^{-1}, intermediately degradable fraction (CB1) which is starch and pectin with an intermediate degradation rate of 0.20–0.50 h^{-1}, a slowly degradable fraction (CB2) which is available cell wall with a slow degradation rate of 0.02–0.10 h^{-1}, and an unfermentable fraction (CC) which is the unavailable cell wall.[20]

Rumen Degradation Kinetics

In situ rumen degradation kinetics of DM, CP, OM and NDF were determined using the first-order kinetics equation described by Ørskov and McDonald[21] and modified by Robinson *et al.*[22] and Dhanoa[23] to include lag time:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)},$$

where R(t) = residue present at t h incubation (g kg^{-1}); S = soluble fraction (g kg^{-1}); U = undegradable fraction (g kg^{-1}); D = potentially degradable fraction (g kg^{-1}); T0 = lag time (h); and K_d = degradation rate (%/h). The results were calculated using the NLIN (nonlinear) procedure of SAS (SAS Institute, Cary, NC) with iterative least squares regression (Gauss-Newton method).

Based on the nonlinear parameters estimated by the above equation (*S*, *U* and Kd), rumen-degraded feed CP (RDP), rumen undegraded CP (RUP) were predicted according to the NRC 2001[17] model as:

$$RDP\ (g\ kg^{-1}) = S + (D \times K_d) / (K_p + K_d)$$

$$RUP\ (g\ kg^{-1}) = U + (D \times K_d) / (K_p + K_d)$$

where $D = 100 - S - U$ (g kg^{-1}) and K_p is the estimated rate of outflow of digesta from the rumen (% h^{-1}), which was assumed to be 6% h^{-1}.

Estimation of the Intestinal Digestibility of Rumen Undegraded Crude Protein (RUP)

Intestinal digestibility of rumen undegraded feed protein was determined according to the protocol for ruminants.[24] Dried ground rumen residues containing 15 mg of N after 12 h of ruminal incubation were exposed for 1 h to 10 mL of 0.05 mol L^{-1} HCl solution containing 1 g L^{-1} pepsin. The pH was then neutralised with 0.5 mL of 0.5 mol L^{-1} NaOH and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg of pancreatin, which were added to the solution and incubated at 38°C for 24 h. After 24 h incubation, 3 mL of a 100% (wt/vol) trichloroacetic acid (TCA) solution was added to precipitate undigested

proteins[24]. The samples were centrifuged and the supernatant was analyzed for N (Kjeldahl method, AOAC 984.13). Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the 12 h residue sample.

Statistical Analysis

Chemical profile, protein and carbohydrate fractions, estimated energy values, *in situ* rumen degradation kinetics and *in vitro* intestinal digestibility of rumen undegraded feed crude protein.

Statistical analyses were performed using the MIXED procedure of SAS 9.2. (SAS Institute, 1999). Data were analyzed with a CRD model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where, Y_{ij} was an observation of the dependent variable $_{ij}$; m was the population mean for the variable; T_i was the effect of feed source, as a fixed effect, batch was as replication and e_{ij} was the random error associated with the observation $_{ij}$.

The significance of differences between means was assessed using Tukey's test. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

Results and Discussion

Chemical Composition

The chemical profiles of canola meal yellow-seeded (CM_Y), brown-seeded (CM_B) and canola presscake brown-seeded (CPC_B) are presented in Table 1. Significant differences between CM_Y and CM_B were found for the basic nutrients except ash content (P>0.05) and organic matter (P>0.05). CM_Y had lower ash and ether extract (P<0.05) than CM_B. Almost 7 times higher (P<0.05) was found the values of EE for CPC_B compared to the CM_Y and CM_B respectively. In accordance to our study in the literature, is mentioned that since presscake has not passed through the solvent extraction process, the oil content is higher while crude protein is lower than in canola meal.[6,7] Moreover, according to Thacker and Petri[25], in Canada cold-pressed canola cake, contained 32% CP, 26% NDF, and 20% ADF on a DM basis. Higher values for the same parameters were found in our study. This could be attributed to the different production method of the canola presscake in our study, which was screw-pressing in contrast with their cold-pressed canola cake. Generally, differences in crude protein, amino acids or minerals can be mainly due to seed characteristics as affected by genetic and environmental factors while differences in EE could reflect processing conditions and differences in glucosinolate content of canola meal and canola presscake could reflect cultivar, environmental and processing factors.

For carbohydrate profiles, CM_Y was lower in NDF (23 *vs* 34 %DM P<0.005), ADF (10 *vs* 18 %DM, P<0.05) and ADL (3 *vs* 10 %DM, P<0.05), but higher in NSC (54 *vs* 39 %CHO, P<0.05) than CM_B (Table 1). For the parameters mentioned before, no significant differences observed between CPC_B and CM_B, while higher (P<0.05) were the values for CPC_B compared to CM_Y except the NSC (%CHO) that was lower (P<0.05). It was reported by Simbaya *et al*.[5] that dietary fiber content in yellow-seeded samples was significantly lower, differing by 6 percentage points from that of the brown-seeded types. They supported that such a discrepancy could be explained by environmental conditions, genetic differences, or location as the canola samples used in this study were collected from different canola suppliers and collection dates. Moreover, the lower fiber content of canola yellow-seeded was noted to be a consequence of a bigger seed size, a lower contribution of the hull fraction to the total seed mass, and a lower fiber content of the hull fraction.[4] Moreover, Abraham and Bhatia[42], mentioned that yellow-seeded *Brassica* genotypes have a thinner and translucent seed coat, resulting in a lower hull proportion with a bigger embryo and consequently greater oil and protein contents in the seeds. Also, they noted that the seed coats of black/brown seeded *Brassica* genotypes contains more fiber and less protein than those of yellow seeded genotype.[41,42]

Regarding the protein profile of the feedstuffs used in this study, CM_Y had higher (P<0.05) values for CP, lower (P<0.05) SCP (%CP), ADICP (% CP) and NDICP (%CP) than CM_B (Table 1). Canola brown-seeded presscake had significant lower CP values (34 *vs* 46) compare to CM_Y, while no differences observed with CM_B. Higher were values of CPC_B (P<0.05) for SCP (%CP), NPN (%CP),

NDICP (%CP or %DM) and ADICP (%CP or %DM) in comparison with the CM_Y. Mustafa *et al*.[7] reported that soluble protein content in solvent extracted meal reduces by more than half after toasting, which they attributed to both high moisture and high heat prevailed at that processing stage.

There are a few minor components in canola meal which may have anti-nutrient effects.[1] One group of these components is condensed tannins (CT), which are present in a range of 1.5% to 3.0%, with brown-seeded varieties having higher levels than yellow-seeded.[1] In this study, no significant differences for the CT content were observed among the feedstuffs. However, numerically, CT content was higher for CM_B compare to CM_Y. According to Shahidi *et al*.[26] phenolics compounds (i.e. CT) contribute to dark colour of canola meals. The tendency of tannin content to increase with intensity of seed coat colour was reported by Plahar *et al*.[27] for cowpea and Sedghi *et al*.[28]. Furthermore, Akhov *et al*.[29] concluded that the canola seed coat pigmentation was attributed to oxidised CT and that their accumulation was most robust in young seed coats of black-seeded line and only very little in yellow-seeded line. This could be another reason for the numerically lower values of CT content observed for CM_Y.

From the comparison of CPC_B, with the same brown- seeded, canola meal, the last had higher CT content ($P>0.05$). In the literature is mentioned that, heat treatment induced the formation of insoluble complexes of tannins with other seed components or changes in their chemical reactivity.[30] This may resulted in a reduce of their extractability by the analytical method. So it was possible that the level of the extractable condensed tannins was low during the processing stage where presscake was produced (before the solvent-extraction).

Protein and Carbohydrate Fractions

Protein and carbohydrate fractions partitioned by the CNCPS system[20] are shown in Table 2 and Table 3. The true protein fractions (PB1 and PB3) and the non-protein PA fraction between CM_Y and CM_B were very similar ($P>0.05$). However, CM_Y was higher in the intermediately degradable protein fraction (PB2: 75 *vs* 65 %CP) and lower in the unavailable CP fraction (PC: 0.2 *vs* 3 %CP) compared to the CM_B. No significant differences, were found in this study, between CPC_B and both two CM varieties for the rapidly (PA1) and slowly (PB3) degradable protein fraction. CPC_B was lower in the intermediately degradable CP fraction (P2) and higher in the PA and PC fraction (Table 2). The non-protein nitrogen and the rapidly degradable true protein fractions denature at lower heat inputs and become intermediately or slowly degradable true protein fractions based on the level of heat input. The slowly degradable protein fraction responds at higher heat inputs and usually becomes unavailable (heat damage) protein via Maillard reaction. Moreover, the fraction PC is not consider degradable in the rumen and the protein in this fraction is found to be bound to feed constituents (i.e. lignin, tannins) and is not available to the animal. From this study was indicated that PC fraction (expressed as %CP or %DM) for CM_Y was markedly lower than the other two samples used. This result, in relation with the high value for CM_Y intestinal protein digestibility, obtained *in vitro*, implies that yellow-seeded canola meal is an excellent source of intestinal digestible proteins for ruminants. The PC fraction is represented the acid detergent insoluble protein (ADIP). PC fraction contains proteins associated with lignin, tannins and heat damaged proteins such as Maillard products and assumed to be indigestible. The lower value of PC for CM_Y indicates that proteins, compared with CM_B, was affected less by the processing method. Moreover, the degradation rates of PB1, PB2 and PB3 fractions were reported to be 135% to 350%, 3% to 6% and 0.05% to 0.55%/h respectively.[20] Based on these rates and the distribution of the three sub-fractions in each feedstuff, CM_Y was expected to show comparatively faster ruminal degradability compared to CM_B and CPC_B. Indeed, EDCP was lower (42 %CP) compare to CM_B and CPC_B (51 %CP and 53 %CP respectively) (Table 5).

For all the carbohydrate fractions (Table 3), CM_Y was markedly higher in comparison with the CM_B. In our study, it was found that the CA fraction, represent the degradable soluble sugars, was higher for CM_Y compare to CM_B. This is in accordance with the results reported by Slominski *et al*.[4] and Simbaya *et al*.[5] who compared the yellow-seeded with brown-seeded varieties of canola. On one hand, the carbohydrate fractions (CA, CB2) of CM_B were higher than CPC_B. On the other hand, CPC_B found to have significant lower values for all the carbohydrate fractions CA, CB2 and CC than CM_Y. These results indicated that CPC_B contained lower amounts of rapidly degradable soluble sugars (CA)

and a lower slowly degradable fraction associated with the plant cell wall but available depending on the rate of rumen turnover and also lower undegradable cell wall.

Energy Values

Truly digestible nutrients and energy content of canola meals and canola presscake are presented in Table 4. CM_Y was higher (P<0.05) in total digestible CP and total digestible NFC, while it was lower in total digestible FA, compared to CM_B. Moreover, our results indicated that CPC_B had lower (P<0.05) value for total digestible NFC, total digestible CP, total digestible NDF and higher (P<0.05) value for total digestible FA. Gross energy (GE) was not different between the two varieties of CM. Significant higher was the GE value found for CPC_B compared to both varieties of canola meal (5 *vs* 4 mean value for CM). Total digestible nutrients content was numerically higher for CPC_Y but no significant differences observed among the three feedstuffs used in this study. Energy values (DE_{3X}, ME_{3X} for dairy; ME, NE_m and NE_g for beef cattle) were different (P<0.05) between CM_Y and CM_B and higher for CM_Y. Similar (P>0.05) were the values (DE_{3X}, $ME_{3X,}$ NE_{L3x}) between CM_Y and CPC_B. Predicted DE values for canola meal in our study (DE_{1x} or DE_{3x}) were similar with those obtained by Zinn[31] in feedlot cattle fed with high energy diet. In the literature, it is noted that EE is an important determinant of energy value; greater residual oil in the cake increased the DE and NE content. However, this was not the case in our study as the higher values EE observed for CPC_B were not resulted in higher values of DE or NE content. According to Keith and Bell[6], GE of CM averages 4.4 ± 0.02 Mcal/kg. The values obtained in our study are within this range or even slightly higher for CM_Y and CM_B respectively (Table 4). Variation in GE content of canola meal can be due to differences in lipid, protein and fiber contents.[32] Research studies have found that high concentration of fibre in canola meal diluted the digestible nutrients[1] and reducing its metabolizable energy. This is in agreement with the results found in our study. The ME_{3x} for dairy cattle of CM_B was predicted by using the NRC 2001 and was found to be lower (P<0.05) compare to CM_Y or CPC_B. This could be explained by the higher NDF content found for CM_B.

Degradation Kinetics

The effect of processing method and variety on rumen fractions, rate of degradation and effective degradability of nutrients is presented in Table 5. Comparing CM_Y with CM_B, no significant differences were observed in terms of the effective degradability of DM (EDDM). CM_Y was higher (P< 0.05) in soluble (*S*) and degradable (*D*) fraction and lower (P> 0.05) in K_d degradation rate. There was no significant difference for the *S* fraction between CM_B and CPC_B. The *D* fraction of DM in canola yellow seeded (88 %/h) was significantly higher than CPC_B (71 %/h). No significant differences in DM degradation were observed among the two CM varieties and CPC; however numerical differences indicated that DM degradation of CM_Y was faster and degraded more extensively due to the higher *D* fraction (Table 5). The values of EDDMS and K_d of canola meal were lower than those reported by Heendeniya.[33] Ash content differences between the two CM varieties and CPC were not highly parallel to DM content differences (Table 1) and as a consequence the pattern and changes of OM degradation kinetics were not highly related to those of DM and were even lower (Table 5).

Canola presscake, although that was not significant, had higher in *sacco* soluble CP fraction than CM_Y or CM_B. The EDCP (%CP) of CM_Y was significant lower compare to that of CM_B (Table 5). The lower solubility of CM, compared to CPC, can be attributed to heating of CM in the desolventizer-toaster step during oil extraction. Consistent with our results, Mustafa *et al.*[7] reported a higher protein solubility for CPC than for CM which resulted is an also higher CP ruminal degradability. In contrast with our study, Mustafa *et al.*[7] found lower values for effective CP degradability for yellow and brown-seeded). However, higher values for canola meal, than those obtained in our study, observed by Woods *et al.*[34] The lower values in our study, might be due to the fact that they used nylon bags with a higher pore size (50 μm). Higher values for the CP effective degradability of CM and were also observed by Jones *et al*.[9] This difference between the studies is might be due to the experimental procedure. For example, their feed samples were incubated in the rumen less hour compare to our study and their calculations were include a ruminal flow k_p of 5%/h than 6%/h in our study.

The effective degradability of NDF (%DM) and the ruminal degradation rate K_d were not different among the feedstuffs used in this study (P>0.05). However, when degradability of NDF expressed as %NDF, higher values were observed (P<0.05) for CM_Y compare to CM_B or CPC_B (Table 5). This was closely related to the significantly higher values of CM_Y for the *S* fraction compare to the CM_B and CPC_B. No significant difference was detected between CM_B and CPC_B in terms of EDNDF (% NDF). The degradability of NDF depends on proportion of its main components i.e. cellulose, hemicellulose and lignin. Lignin is known to be highly indigestible, as well as render cellulose and hemicellulose that are associated with it, indigestible.[35] In our study the ADL and cellulose content of the CM_Y variety was lower (P<0.05) compare to CM_B and CPC and this could be attributed to a higher EDNDF (%NDF). Mustafa[36] who studied a low fiber canola meal, found slightly higher degradation rate (8.31%/h) for NDF and higher EDNDF value at 5%/h K_p, compared to our CM_Y (similar NDF). The K_d value represents the overall degradation rate of PB fraction of the crude protein and would depend on make-up of PB fraction since its different sub-fractions (i.e. PB1, PB2, PB3) are having different rates of degradation. Hence, the differences in make-up of PB-fraction in the different ingredients could be attributed partly to differences in K_d values for protein observed in this study. It should be noted that for all, DM, OM and CP, the rate of degradation (K_d) was significant different (P<0.05) between the two varieties of CM but also between CM and CPC. The difference obtained between CM_Y and CM_B can be due to difference chemical profile of these two varieties (i.e. higher NDF %DM content for CM_B). However, the reason for the higher values found for CPC can be related, as already have been mentioned, to the effect of the stage of processing.

It is generally accepted heat treatment reduces the reduction of the DM and CP degradation and this effect being partly related to the blocking of sites reactive for microbial proteolysis enzymes and partly to the reduction of protein solubility[37] and increase the supply of dietary protein to the duodenum.[38,39]

Intestinal Digestibility of Rumen Undegradated Rumen Protein

Results of the intestinal digestibility estimation are presented in Table 6. *In vitro* intestinal digestibility for CM_Y was higher (P<0.05) than that obtained for CM_B. Canola presscake was an inferior (P<0.05) source of RUP compare to both two varieties of CM. In the literature is referred that heating of canola meal[40,10] and canola seed[8] shown to decrease protein degradability in the rumen without affecting intestinal digestibility. This was also supported by our results, cause although the protein degradation was quite low this was not negatively affect digestibility in the small intestine. The lower values for CPC_B could be partly explained due to the production conditions. CPC_B was not exposed to the high temperature of the desolventizer-toaster, which can decrease degradation of dry matter and crude protein by blocking reactive sites for microbial proteolytic enzymes and increase the supply of dietary protein to the duodenum.[39] The value for CM_B obtained *in vitro* in our study, was similar to that reported by Zinn[31] *in vivo*. They found that the postruminal N digestion for a solvent-extracted CM (CP: 40 %DM) was 72 *vs* 74 in our study. However, a lower value (62.7 % intake) was obtained by Khorasani *et al.*[41] for canola meal and the digestibility of CP in the small intestine. The difference might be due to the fact that we used a *in vitro* method for the determination of intestinal digestibility while the perform *in vivo* trial with cannulated calves.

It should be also noted that CP disappearance in the intestine and total tract of the ruminant is negatively correlated with ADIN content of the feedstuff. Moshtaghi Nia and Ingalls[40] reported a negative relationship between total tract disappearance of N and ADIN level of autoclaved canola meal (r = -0.81). Their results support findings of Van Soest and Mason[14] who reported a high positive correlation between faecal N and ADIN and a negative correlation between N digestibility and ADIN on forages. Results of our study were in agreement with previous ones, as a higher intestinal digestibility was observed for CM_Y in relation to the lower (P<0.05) ADIP value.

Conclusion

This study investigated the effect of processing condition on the nutritive value of canola meal in comparison with a canola presscake, and the effect of variety between a yellow-seeded and brown-seeded canola meal. The processing method, up to the stage of solvent-extraction where the production of canola

presscake occurs, had significant influence on its chemical composition, effective protein degradability and protein and carbohydrates fractions. This work confirmed that, since presscake has not passed through the solvent extraction process, had a lower protein but higher oil content than canola meal and is a potential great energy supplement source for ruminants.

The comparison between yellow-seeded and brown-seeded canola meals showed that the first one had higher crude protein and low fiber content. The same observed also for its digestible and metabolizable energy values. It is known that the lower ruminal protein degradation could be beneficial, only is it is associated with the high digestibility in small intestine. This was the case for yellow-seeded canola meal, as its in vitro protein intestinal digestibility was markedly higher than that of brown-seeded one. Nowadays, with yellow-seeded canola, there is potential, as also showed in this study, for much improved quality of the meal as an animal protein feed and be an alternative to the premium protein animal feed, the soybean meal. In plant breeding, selection programmes are geared towards yellow seeded varieties in order to improve the nutritive value of canola meal.

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Table 1. Chemical profiles: Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*).

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)			CM *vs* CPC P value
Basic chemical (g kg^{-1})						
DM	89.0^{b}	88.7^{b}	94.4^{a}	0.35	0.002	0.001
Ash	72.2^{b}	79.0^{a}	64.1^{c}	1.08	0.005	0.003
EE	22.8^{b}	40.4^{b}	147.2^{a}	14.42	0.016	0.007
OM	927.9^{b}	921.0^{c}	936.0^{a}	1.08	0.005	0.003
Carbohydrate profile						
NDF (g kg^{-1}DM)	237.3^{b}	342.0^{a}	337.9^{a}	5.77	0.002	0.007
ADF (g kg^{-1}DM)	103.5^{b}	182.9^{a}	193.4^{a}	3.08	< 0.001	0.001
ADL (g kg^{-1}DM)	34.7^{b}	101.4^{a}	107.2^{a}	2.82	0.001	0.002
Hemicellulose (g kg^{-1}DM)	133.8^{a}	159.1^{a}	144.5^{a}	8.57	0.259	0.865
Cellulose (g kg^{-1}DM)	68.9^{b}	81.5^{a}	86.2^{a}	1.70	0.012	0.013
CHO (g kg^{-1}DM)	435.6^{b}	480.8^{a}	443.2^{b}	5.25	0.017	0.101
NSC (g kg^{-1} CHO)	548.5^{a}	397.9^{b}	387.0^{b}	6.20	0.001	0.016
NSC (g kg^{-1}DM)	237.4^{b}	342.1^{a}	338.0^{a}	5.77	0.002	0.007
NDF_n (g kg^{-1}DM)	196.7^{b}	289.6^{a}	271.8^{b}	4.44	0.001	0.013
ADF_n (g kg^{-1}DM)	102.4^{b}	170.5^{a}	157.1^{a}	2.88	0.001	0.010
Protein Profile						
CP (g kg^{-1}DM)	469.5^{a}	399.8^{b}	345.6^{b}	11.31	0.010	0.008
SCP (g kg^{-1}DM)	763.0^{a}	873.0^{a}	824.0^{a}	5.67	0.479	0.093
SCP (g kg^{-1} CP)	162.3^{b}	218.2^{a}	238.3^{a}	8.51	0.017	0.019
NPN (g kg^{-1}DM)	54.1^{b}	61.2^{ab}	62.7^{a}	4.08	0.402	0.389
NPN (g kg^{-1} CP)	115.3^{b}	152.9^{ab}	181.5^{a}	7.62	0.012	0.015
NPN ((g kg^{-1} SCP)	710.7^{a}	699.8^{a}	762.2^{a}	59.18	0.247	0.124
NDICP (g kg^{-1}DM)	40.7^{b}	52.5^{ab}	66.2^{a}	2.31	0.010	0.062
ADICP (g kg^{-1}DM)	1.2^{c}	12.4^{b}	36.4^{a}	0.27	0.001	0.001
NDICP (g kg^{-1} CP)	86.6^{c}	131.3^{b}	191.6^{a}	1.75	0.001	0.001
ADICP (g kg^{-1} CP)	2.35^{c}	31.0^{b}	105.6^{a}	3.50	0.001	0.002
Condensed Tannins						
CT (g kg^{-1}DM)	5.7^{a}	13.5^{a}	1.0^{a}	2.15	0.058	0.047

SEM= Standard error of mean; $^{a, b, c}$ Means with different letters within the same row differ ($P < 0.05$).
DM: Dry Matter; OM: Organic Matter; EE: Ether Extract (crude fat); NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; CHO: Carbohydrate; NSC: Non-Structural Carbohydrate; NDF_n: Nitrogen free Neutral Detergent Fiber; ADF_n: Nitrogen free Acid Detergent Fiber; CP: Crude Protein; SCP: Soluble Crude Protein; NPN: Non-protein Nitrogen; NDICP: Neutral Detergent Insoluble Crude Protein; ADICP: Acid Detergent Insoluble Crude Protein; CT: Condensed Tannins.

Table 2. Cornell Net Carbohydrate and Protein system (CNCPS): Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*)

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)			CM *vs* CPC P value
Total crude protein (g kg^{-1}DM)	469.5[a]	399.8[b]	345.6[b]	11.31	0.010	0.008
Protein fractions (g kg^{-1} CP)						
PA	115.3[b]	152.9[ab]	181.5[a]	7.62	0.019	0.015
PB1	47.1[a]	65.3[a]	56.9[a]	5.36	0.199	0.922
PB2	751.1[a]	650.6[b]	570.2[c]	9.46	0.002	0.002
PB3	84.3[a]	100.3[a]	86.0[a]	3.63	0.093	0.255
PC	2.4[c]	31.0[b]	105.6[a]	3.48	0.001	< 0.001
True Protein (g kg^{-1} CP)	882.4[a]	816.1[b]	713.1[c]	8.57	0.002	0.001
Protein fractions (g kg^{-1} TP)						
PB1	53.4[a]	80.0[a]	79.7[a]	6.74	0.108	0.213
PB2	851.2[a]	797.2[a]	799.8[a]	11.07	0.007	0.169
PB3	95.5[b]	122.9[a]	120.1[ab]	4.56	0.041	0.133
Protein fractions (g kg^{-1} DM)						
PA	54.1[a]	61.2[a]	62.7[a]	4.07	0.399	0.383
PB1	22.1[a]	19.8[a]	26.1[a]	2.56	0.346	0.264
PB2	352.6[a]	260.0[b]	196.9[c]	4.67	0.004	<0.001
PB3	39.6[a]	40.1[a]	29.9[a]	2.27	0.082	0.037
PC	1.2[c]	12.4[b]	36.4[a]	0.27	< 0.001	< 0.001

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).
True protein = PB1 (g kg^{-1} CP) + PB2 (g kg^{-1} CP) + PB3 (g kg^{-1} CP). TP: true protein; PA: fraction of CP that is instantaneously solubilized at time zero, calculated as NPN; PB1: rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN; PB2: intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC; PB3: slowly degradable protein fraction, calculated as NDICP minus ADICP; PC: fraction of undegradable protein, calculated as ADICP. It contained the proteins associated with lignin and tannins and/or heat-damaged proteins such as Maillard reaction products.

Table 3. Cornell Net Carbohydrate and Protein system (CNCPS): Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*)

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)			CM *vs* CPC P value
Carbohydrates fractions (g kg^{-1} CHO)						
CHO	43.6[b]	48.0[a]	44.3[b]	0.52	0.017	0.101
CA	54.9[a]	39.8[b]	38.7[b]	0.62	< 0.001	0.002
CB2	26.0[a]	9.6[b]	3.5[b]	2.22	0.012	0.013
CB1	ND	ND	ND			
CC	19.1[b]	50.6[a]	58.1[a]	1.87	0.001	0.002
Carbohydrates fractions (g kg^{-1} DM)						
CA	23.9[a]	19.1[b]	17.2[b]	0.378	0.002	0.003
CB2	11.3[a]	4.6[b]	1.6[b]	1.033	0.015	0.015

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).
CHO = 100 – crude protein – ether extract – ash. CHO: carbohydrate; CA: fraction of total carbohydrate with a rapidly K_d (300%/h) and is degradable soluble sugars and organic acids; CB1: fraction of total carbohydrate with an intermediately degradable fraction (K_d = 20-50%/h) consisting of starch and pectin, CB2: fraction of total carbohydrate with a slow K_d (2-10%/h) and is available cell wall; CC: fraction of total carbohydrate and is unavailable cell wall and not fermented. CC is calculated as 0.024 times ADL, CA is calculated as NFC minus CB1.

Table 4. Truly digestible nutrients and energy content: Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus)*

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)			CM *vs* CPC P value
Truly digestible fractions (g kg^{-1}DM) (NRC 2001)						
tdNFC	234.5[a]	187.5[b]	168.1[b]	3.91	0.027	0.003
tdCP	469.0[a]	364.9[b]	331.1[b]	11.30	0.008	0.005
tdFA	12.8[b]	30.4[b]	137.2[a]	14.42	0.016	0.007
tdNDF	83.1[a]	71.1[ab]	57.6[b]	4.25	0.054	0.033
Total digestible nutrients (g kg^{-1}DM) (NRC 2001)						
TDN_{1x}	74.5[ab]	65.2[ab]	79.5[a]	1.43	0.030	0.024
Gross Energy (MJ kg^{-1}) (Bob-calorie-meter)	18.4[b]	18.5[b]	21.4[a]	0.06	0.007	0.003
Predicted digestible energy value at maintenance level intake (MJ/kg DM; NRC 2001)						
DE_{1x}	3.8[a]	3.3[b]	3.8[a]	0.06	0.018	0.046
Predicted energy value at production intake level (3X) for dairy cattle (MJ/kg DM; NRC 2001)						
DE_{3x}	3.5[a]	3.0[b]	3.5[a]	0.06	0.017	0.046
ME_{3x}	3.1[a]	2.6[b]	3.1[a]	0.01	0.019	0.034
NE_{L3x}	2.0[ab]	1.7[b]	2.1[a]	0.06	0.026	0.045
Predicted energy value for beef cattle at intake level 3X (MJ/kg DM; NRC, 1996)						
ME	3.1[a]	2.3[b]	3.1[b]	0.05	0.017	0.044
NE_m	2.1[a]	1.8[b]	2.1[a]	0.04	0.014	0.037
NE_g	1.5[a]	1.2[b]	1.5[a]	0.04	0.018	0.049

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

tdNFC: digestible non-fiber carbohydrate; tdCP: digestible crude protein; tdNDF: digestible neutral detergent fiber; tdFA: digestible fatty acid ; TDN_{1X}: total digestible nutrients at maintenance estimated from NRC dairy model 2001; DE_{3X}: digestible energy three times maintenance estimated from the NRC dairy model 2001; ME_{3X}: metabolizable energy at three times maintenance estimated from the NRC dairy model 2001; NEL_{3X}: net energy for lactation at three times maintenance estimated from the NRC dairy model 2001; ME: metabolizable energy estimated from the NRC beef model 1996; NE_m: net energy for maintenance estimated from the NRC beef model 1996; NE_g: net energy for growth estimated from the NRC beef model 1996. DE_{1X}: Digestible energy at maintenance

Table 5. *In situ* rumen degradation characteristics of dry matter, crude protein, organic matter and neutral detergent fiber. Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus)*

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)			CM *vs* CPC P value
In situ rumen dry matter degradation characteristics						
S (g kg^{-1}DM)	97.7^{a}	77.0^{b}	72.3^{b}	2.95	0.017	0.025
D (g kg^{-1}DM)	881.6^{a}	729.8^{b}	714.5^{b}	14.06	0.006	0.013
K_d (%/h)	5.1^{c}	7.8^{b}	9.3^{a}	0.23	0.002	0.002
RUDM (g kg^{-1}DM)	496.8^{a}	509.7^{a}	494.0^{a}	5.17	0.219	0.240
EDDM (g kg^{-1}DM)	506.5^{a}	503.3^{a}	490.4^{a}	5.17	0.219	0.240
In situ rumen organic matter degradation characteristics						
S (g kg^{-1} OM)	151.7^{a}	135.4^{a}	80.6^{b}	7.92	0.016	0.007
D (g kg^{-1} OM)	830.1^{a}	664.2^{b}	703.5^{b}	15.60	0.010	0.106
K_d (%/h)	5.0^{c}	7.7^{b}	9.0^{a}	0.20	0.002	0.002
RUOM (g kg^{-1} OM)	471.8^{b}	490.9^{ab}	497.6^{a}	3.32	0.025	0.029
RUOM (g kg^{-1} DM)	437.8^{b}	452.1^{ab}	465.7^{ab}	2.94	0.016	0.010
EDOM (g kg^{-1} OM)	588.3^{a}	509.1^{ab}	502.5^{b}	3.33	0.025	0.029
EDOM (g kg^{-1} DM)	490.1^{a}	468.9^{b}	470.3^{b}	3.32	0.034	0.109
In situ rumen crude protein degradation characteristics						
S (g kg^{-1} CP)	24.2^{a}	47.0^{a}	50.7^{a}	8.86	0.219	0.258
D (g kg^{-1} CP)	975.9^{a}	871.2^{b}	818.9^{b}	9.71	0.003	0.003
K_d (%/h)	4.23^{c}	6.935^{b}	8.7^{a}	0.10	< 0.001	< 0.001
RUCP (g kg^{-1} CP)	572.4^{a}	486.1^{b}	464.7^{b}	4.38	< 0.001	0.001
RUCP (g kg^{-1} DM)	250.2^{a}	185.3^{b}	167.2^{b}	3.92	0.001	0.002
EDCP (g kg^{-1} CP)	427.5^{b}	513.9^{a}	535.4^{a}	4.38	< 0.001	0.001
EDCP (g kg^{-1}DM)	186.9^{a}	195.9^{a}	192.5^{a}	1.67	0.069	0.629
In situ rumen neutral detergent fiber degradation characteristics						
S (g kg^{-1} NDF)	53.2^{a}	16.6^{ab}	16.6^{b}	7.0^{b}	0.035	0.045
D (g kg^{-1} NDF)	884.5^{a}	535.4^{b}	627.9^{b}	39.85	0.018	0.191
K_d (%/h)	4.0^{a}	5.1^{a}	4.9^{a}	0.72	0.612	0.746
T0 (%/h)	2.4^{a}	1.4^{a}	1.7^{a}	1.70	0.914	0.923
RUNDF (g kg^{-1} NDF)	595.2^{b}	739.0^{a}	717.4^{a}	7.26	0.002	0.011
RUNDF (g kg^{-1} DM)	102.2^{b}	189.5^{a}	218.1^{a}	5.32	0.001	0.002
EDNDF (g kg^{-1} DM)	404.8^{a}	261.1^{b}	282.7^{b}	7.26	0.002	0.011
EDNDF (g kg^{-1}DM)	69.45^{a}	66.9^{a}	85.9^{a}	3.67	0.064	0.029

SEM= Standard error of mean; $^{a, b, c}$ Means with different letters within the same row differ ($P<0.05$).
S = potential soluble fraction in the *in situ* ruminal incubation; D = potentially degradable fraction in the *in situ* ruminal incubation; K_d = degradation rate (%/h); RUDM: Rumen Undegraded Dry Matter; EDDM: Effectively degraded dry matter; RUOM: Rumen Undegraded Organic Matter: EDOM: Effectively degraded Organic Matter; RUCP: Rumen Undegraded Crude Protein; EDCP: Effectively degraded Crude Protein; RUNDF: Rumen Undegraded Neutral Detergent Fiber; EDNDF: Effectively Degraded Neutral Detergent Fiber

Table 6. *In vitro* crude protein digestibility in the small intestine (IVCPD), on the 12h residue determined using three fistulated dairy cattle: Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus)*

	Canola treatment					Contrast
Item	CM-Yellow *B. Juncea* (n=2)	CM-Brown *B. Napus* (n=2)	CPC-Brown *B. Napus* (n=2)	SEM	P value	CM *vs* CPC P value
In vitro crude protein digestibility in the small intestine (g kg^{-1} CP in ruminally incumated residues)						
IVCPD	903.8[a]	749.1[b]	601.4[c]	24.40	0.007	0.005

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).
IVCPD: *in vitro* crude protein digestibility in small intestine.

Project 2: METABOLIC CHARACTERISTICS OF THE PROTEINS IN YELLOW-SEEDED AND BROWN-SEEDED CANOLA MEAL AND PRESSCAKE IN DAIRY CATTLE: COMPARISON OF THREE SYSTEMS (PDI, DVE, NRC) IN NUTRIENT SUPPLY AND FEED MILK VALUE (FMV) (COMPLETED) *

A version of this project has been published: Katerina Theodoridou and Peiqiang Yu. 2013. Metabolic Characteristics of the Proteins in Yellow-Seeded and Brown-Seeded Canola Meal and PressCake in Dairy Cattle: Comparison of Three Systems (PDI, DVE, NRC) in Nutrient Supply and Feed Milk Value (FMV). Journal of Agricultural and Food Chemistry (USA). 61: 2820−2830 (DOI: 10.1021/jf305171z) (*as supervisor, PI, corresponding author).

Project Details

Abstract

To our knowledge, there is little research on metabolic characteristics of the protein in newly developed yellow-type and brown type of Canola meal and canola presscake. The objectives of this study were to (1) identify differences in the metabolic characteristics of the protein among yellow-seeded (*Brassica juncea*) and brown-seeded (*Brassica napus*) canola meal and brown-seeded (*Brassica napus*) canola presscake modeled for dairy cattle, (2) determine the extent of ruminal and intestinal digestion and absorption of the protein, (3) determine feed milk value and (4) compare three evaluation systems in modeling nutrient supply to dairy cattle, namely, the DVE/OEB system (DVE = truly absorbed protein in the small intestine; OEB = degraded protein balance), the National Research Council (NRC) 2001 model and the PDI system (Protein truly Digestible in the small Intestine). Comparison was made in terms of 1) ruminally synthesised microbial protein, 2) truly absorbed protein in the small intestine, 3) endogenous protein; 4) total metabolizable protein; and 5) degraded protein balance. The results showed that there were significant differences in the truly absorbed protein supply, protein degraded balance and feed milk value ($P < 0.05$) among the different types of canola meal. Yellow-seeded canola meal had significantly higher ($P < 0.05$) intestinal digestibility of rumen undegraded crude protein (%dRUP) than brown-seeded canola meal and presscake (%dRUP: 90 vs. 75 and 60%, respectively). Yellow-seeded canola meal also had higher ($P < 0.05$) total metabolizable protein predicted by all three models (DVE: 312 vs. 192 and 128 g/kg DM; MP: 287 vs. 193 and 168 g/kg DM, and PDIA: 264 vs. 168 and 137 g/kg DM, respectively), lower ($P<0.05$) degraded protein balance (OEB: 84 vs. 104 and 102 g/kg DM; DPB: 49 vs. 60 and 57 g/kg DM, respectively) and higher ($P < 0.05$) feed milk value (6.3 vs. 3.9 and 2.6 kg milk/kg feed, respectively) than the brown-seeded canola meal and presscake. In the model comparison, the supply of endogenous protein predicted by the DVE/OEB system was higher ($P<0.05$) than that predicted by NRC-2001 model. Moreover, a high proportion of the variability in truly absorbed rumen-undegraded feed protein in the small intestine and the total metabolisable protein predicted by DVE/OEB system was found that can be accounted for the equivalent parameters predicted by the NRC-2001 model. The truly absorbed rumen-synthesized microbial protein values predicted from PDI system were 19% lower than those predicted from the DVE/OEB system. Between the two latest mentioned models, no differences were detected in truly absorbed rumen-undegraded feed protein; microbial protein supply based on available energy and degraded protein balance. All the parameters predicted by the PDI system can be accounted for by the equivalent parameters predicted by the DVE/OEB system. When comparing PDI system and NRC-2001 model, the overall means for microbial protein supply based on energy and truly absorbed rumen-synthesized microbial protein were found to be lower than those predicted by the NRC-2001 model. Although the factors used in quantifying calculations as well as the evaluation system's concepts differ among each other, all three protein evaluation systems employed in this study, efficiently

predict the potential nutrient supply to the animal from feedstuffs as affected by processing. In conclusion, the yellow-seeded canola meal provided highest total metabolizable protein and lowest degraded protein balance.

Keywords: Canola, Protein metabolic characteristics, Nutrient supply modeling

Introduction

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in the 1970's. Unlike with traditional rapeseed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30 μmol).[1] Canola meal includes the newly developed yellow-seeded and brown-seeded varieties; hulls from newly developed yellow-seeded have been reported to have lower fiber content compared to those from brown-seeded types.[2-4] The intermediate product in the manufacturing process of canola oil and canola meal is called canola presscake.[5]

In ruminants, canola co-products are good protein sources with high protein quality. However, metabolizable protein information are lacking in canola co-products, particularly newly developed yellow-type of canola co-products. The metabolizable protein value is contributed from three sources: absorbed rumen bypassed protein, absorbed microbial protein synthesis and indigenous protein sources which are important in ruminant nutrient supply.

Ruminants can convert feeds into animal products under widely varying conditions worldwide thus there is a need to systematically evaluate the nutritive value of each feed. Improving ruminant nutrition could be pursuit by enhanced productivity, while further improvements in ruminant production efficiency will result from the use of models, the latest models are able to predict nutrient requirements and feed utilization in specific production settings.

In terms of protein nutrition, the level of microbial protein synthesis and the amount of digestible protein in the intestine are important determinants of the response and efficiency with which dietary nitrogen is used for milk production. These points are taken into account in the most advanced protein evaluation systems, such as the NRC-2001 model,[7] DVE/OEB system,[11] and PDI system.[16]

Objective

So far, little research has been conducted to determine metabolic characteristics of the protein, potential nutrient supply and feed milk value of canola co-products by employing and comparing different evaluation systems. Although the principles of these models (DVE/OEB, PDI, and NRC-2001) are similar, some of the factors used in quantifying calculations and some concepts differ. The objectives of this study were to (1) identify differences in the metabolic characteristics of the protein and energy among yellow-seeded (*Brassica juncea*) and brown-seeded (*Brassica napus*) canola meal and brown-seeded (*Brassica napus*) canola presscake modeled for dairy cattle, (2) determine the extent of ruminal and intestinal digestion and absorption of the protein, (3) determine feed milk value and (4) compare three evaluation systems in modeling nutrient supply, namely, the DVE/OEB system (DVE = truly absorbed protein in the small intestine; OEB = degraded protein balance), the National Research Council (NRC) 2001 model and the PDI system (Protein truly Digestible in the small Intestine).

Material and Methods

The experiment was approved by the Animal Care Committee of the University of Saskatchewan, and all animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care.[6]

Co-Products from Canola Processing

Different types of Canola meal (CM) and canola presscake (CPC) were used in this study as protein sources. Canola co-products samples of solvent-extracted yellow-seeded (*B. juncea)* canola meal (CM_Y)

and brown-seeded (*B. napus*) canola meal (CM_B) from two different places were obtained from Bunge Altona (Manitoba, Canada) and Lethbridge Research Center (Lethbridge, Ab, Canada). Moreover, brown-seeded (*B. napus)* canola presscake (CPC_B) was produced using a physical press method only and obtained from Milligan Biotech (Foam Lake, SK, Canada).

Animals and Diets

Three dry Holstein cows fitted with a rumen cannula, with an internal diameter of 10 cm (Bar Diamond, Parma, ID), were used for the in situ rumen degradation parameters. Cows were housed in the research barn at the University of Saskatchewan during the whole study period. Cows were given ad libitum access to water and individually fed 15 kg (as fed) of a totally mixed ration twice daily (7.5 kg/feeding) 0800 and 1600 h was formulated according to nutrient requirements of the NRC.[7] The total mixed ration consisted of 57% barley silage, 10% alfalfa hay, 5% dehydrated alfalfa pellets and 28% concentrates (containing barley, wheat, oats, canola meal, soybean meal, wheat dried distillers grains with soluble, corn gluten meal, molasses, golden flakes, canola oil, minerals, and vitamins).

Rumen Incubation Procedure

Seven grams of individual ground samples were weighed into each pre-weighed and numbered nylon bag (10 x 20 cm; Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississauga, ON) with the pore size of 40 μm. These bags were tied about 2 cm below the top, allowing a ratio of sample size to bag surface area of 19 mg/cm^2. Samples were incubated in the rumen for 0, 2, 4, 8, 12, 24, and 48 h. Rumen incubations were performed according to the 'gradual addition/all out' schedule.[8] In this technique, the bags assigned for the longest incubation time (48 h), are put in the rumen first and then after 24 h since the first bags have been incubated in the rumen, the next bags with the next longest incubation time (24 h) are added and so on. The multi-bags for each treatment at each incubation time in each experiment run were 2, 2, 2, 2, 4, 4, and 5 bags for incubation times 0, 2, 4, 8, 12, 24, and 48 h, respectively. The maximum number of bags in the rumen at any one time was 28.[8] Treatment samples were randomly assigned to the three dry rumen fistulated Holstein cows in two experimental runs. After the incubation, the bags were removed from the rumen and rinsed under a cold stream of tap water without detergent to remove excess ruminal contents and subsequently dried at 55°C for 48 h and reweighed to complete the calculation. The 0 h incubation samples were only washed under the same conditions. The dried samples were kept in a refrigerated room (4°C) until chemical analysis was performed. The residues of the nylon bags, from both two experimental runs, were collected according to the sample, incubation time, in situ run and treatment.

Rumen Degradation Characteristics

In situ rumen degradation kinetics of CP were determined using the first-order kinetics equation described by Ørskov and McDonald [9] and modified by Robinson et al. [10] to include lag time:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)},$$

where R(t) = residue present at time h incubation (%); S = soluble fraction (%); U = undegradable fraction (%); D = potentially degradable fraction (%); T0 = lag time (h); and K_d = degradation rate (%/h). The results were calculated using the PROC NLIN (nonlinear) procedure of SAS (SAS Institute, Cary, NC) with iterative least squares regression (Gauss-Newton method).

Based on the nonlinear parameters estimated by the above equation (S, U and K_d), rumen-degraded feed CP (RDP), rumen undegraded CP (RUP) were predicted according to the NRC- 2001 model as:

$$RDP \text{ (g/kg of DM)} = S + (D \times K_d) / (K_p + K_d) \text{ and}$$

$$RUP \text{ (g/kg of DM)} = U + (D \times K_p) / (K_p + K_d),$$

Where, D = 100 − S − U (%) and K_p is the estimated rate of outflow of digesta from the rumen (%/h), which was assumed to be 6%/h. [11]

Intestinal Digestibility of Rumen Undegraded Feed Protein

Intestinal digestibility of rumen undegraded feed protein (**dRUP**) was determined according to

ruminants' protocol.[12] Briefly, dried ground rumen residues containing 15 mg of N after 12 h of ruminal incubation were exposed for 1 h to 10 mL of 0.1 N HCl solution containing 1 g of pepsin/L. The pH was then neutralized with 0.5 mL of 0.5 mol NaOH/L and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg of pancreatin, which were added to the solution and incubated at 38°C for 24 h. After 24 h incubation, 3 mL of a 100% (wt/vol) trichloroacetic acid solution was added to precipitate undigested proteins. The samples were centrifuged and the supernatant was analyzed for N (Kjeldahl method, AOAC 984.13). Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the 12 h residue sample.

Nutrient Supply with the DVE/OEB System

Based on the DVE/OEB system provided by Tamminga et al. in 1994 [11] and in 2007 [13], the detailed explanations and calculation were given in following in order to understand how to calculate and predict protein supply to the small intestine of dairy cows as a result of feeding the above concentrates. The DVE/OEB system constitutes a two part system in which each feed has a DVE and OEB value. The DVE value comprises digestible feed protein, microbial protein, and an endogenous protein loss correction. The DVE value was calculated as DVE = $AMCP^{DVE}$ + $ARUP^{DVE}$ − ENDP, where $AMCP^{DVE}$ is absorbable fraction of microbial crude protein (MCP^{DVE}), $ARUP^{DVE}$ is the absorbable fraction of ruminally undegraded feed protein, and ENDP is a correction factor for endogenous protein lost during the digestion process.

The OEB value or degradable protein balance of a feed is the difference between the potential MCP synthesis based on RDP ($MCP_{RDP}{}^{DVE}$) and the potential MCP synthesis based on energy extracted from anaerobic fermentation (MCP_{FOM}).

Therefore, OEB = $MCP_{RDP}{}^{DVE}$ -MCP_{FOM},

where, MCP_{RDP} was calculated as MCP_{RDP} = CP × [1 - (1.11 × RUP (% CP)/100)]. The factor 1.11 in the formula was taken from the French PDI system [14] and represents the regression coefficient of in vivo on in situ degradation data. [11,13]

Microbial Protein Synthesis in the Rumen and Truly Absorbable Rumen Synthesized Microbial Protein in the Small Intestine. MCP_{FOM} was calculated as MCP_{FOM} = FOM × 0.15,

where, the factor 0.15 means that for each kilogram of rumen fermented OM (**FOM**), 150 g of microbial protein CP is assumed to be synthesized in the rumen.[11]

The FOM in the rumen was calculated as FOM (g/kg of DM) = DOM – Cfat – RUP – RUST – FP, where DOM = digestible organic matter, Cfat = ether extract, RUST = ruminally undegraded feed starch and FP = fermentation products for conserved forages. FP and RUST were assumed to be zero for canola meal and canola presscake. Truly absorbable microbial protein synthesized in the rumen ($AMCP^{DVE}$) was calculated as $AMCP^{DVE}$ (g/kg of DM) = 0.75 × 0.85 × MCP_{FOM} (g/kg of DM), where 0.75 and 0.85 are constants representing the assumed amount and digestibility of the true protein contained in $MCP_{FOM,}$ respectively.[11]

Rumen Undegraded Feed Protein and Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine. The content of truly absorbed rumen undegraded feed protein in the small intestine (**$ARUP^{DVE}$**) is based on the content and digestibility of RUP. The RUP^{DVE} was: RUP^{DVE} (g/kg of DM) = 1.11 × [CP (g/kg of DM) × RUP^{DVE} (% CP)/100], so $ARUP^{DVE}$ was then calculated as $ARUP^{DVE}$ (g/kg of DM) = [dRUP (%) × RUP^{DVE} (g/kg of DM)] /100, where dRUP was estimated according to the method of Calsamiglia and Stern.[12]

Endogenous Protein Losses in the Small Intestine. Endogenous protein losses (**ENDP**) in the small intestine are associated with the amount of undigested dry matter (UDM), which was calculated as UDM (g/kg of DM) = (Ash × 0.35) + [OM - ((OM × dOM)/100)]. In the equation, 0.35 is the constant utilized by CVB, [15] indicating that 35% of ash is not digested, and dOM = OM digestibility after 120 h rumen incubation.[11] According to DVE/OEB, 75 g of protein will be absorbed per kg undigested dry matter

(UDM) to compensate for endogenous losses. Therefore, ENDP was calculated as ENDP (g/kg of DM) = 0.075 ×UDM (g/kg of DM).

Truly Digested and Absorbed Protein in the Small Intestine and the Degraded Protein Balance. Truly digested and absorbed protein in the small intestine (**DVE**) are contributed by 1) feed RUP^{DVE}, 2) microbial protein synthesized in the rumen (MCP_{FOM}), and 3) a correction from ENDP. Therefore the DVE value was calculated as
DVE (g/kg of DM) = $ARUP^{DVE}$ + $AMCP^{DVE}$ – ENDP. The DPB value, which shows the balance between potential microbial synthesis based on rumen degraded protein and potential protein synthesis based on energy extracted during anaerobic fermentation of OM in the rumen, was calculated as DPB^{OEB} (g/kg of DM) = $MCP_{RDP}{}^{DVE}$ – $MCP_{FOM.}$

Nutrient Supply with the NRC-2001 Model

Based on the NRC-2001 model[7], the detailed explanations and calculation were given in following.

Microbial Protein Synthesis in the Rumen. Potential ruminally synthesized microbial CP was calculated as MCP_{TDN} (g/kg of DM) = 0.13 × TDN (discounted) in case that RDP exceeded 1.18 × TDN-predicted MCP (MCP_{TDN}). However, when RDP was less than 1.18 × TDN-predicted MCP (MCP_{TDN}), then MCP was calculated as 0.85 of RDP ($MCP_{RDP}{}^{NRC}$). The factor 0.13 means that 130 g of microbial CP is assumed to be synthesized per kg discounted TDN.

Intestinal Digestion of Feed and Microbial Protein. In NRC-2001, true protein and digestibility of ruminally synthesized microbial CP are assumed to be 800 g/kg therefore the amount of truly absorbed MCP **($AMCP^{NRC}$)** was calculated as $AMCP^{NRC}$ (g/kg of DM) = 0.80 × 0.80 × $MCP_{TDN.}$ Truly absorbed rumen undegraded protein in the small intestine was calculated as $ARUP^{NRC}$ = RUP^{NRC} × dRUP, where dRUP was estimated according to the method of Calsamiglia and Stern.[12]

RumenEndogenous Protein in the Small Intestine. Rumen endogenous protein in the small intestine **(ECP^{NRC})** was calculated as ECP (g/kg of DM) = 6.25 × 1.9 × DM. The 6.25 represents the protein / N conversion factor and 1.9 indicates that 1.9 g of endogenous N is originated from 1 kg of DM. Assuming that 500 g/kg of rumen endogenous CP passes to duodenum and 800 g/kg of rumen endogenous CP is true protein (NRC, 2001), the truly absorbed rumen endogenous protein in the small intestine (AECP) value was calculated as AECP (g/kg of DM) = 0.50 × 0.80 × ECP.

Total Metabolisable Protein. Total metabolisable protein (MP) in the NRC-2001 model is contributed by: (1) ruminally undegraded feed CP (RUP^{NRC}), (2) ruminally synthesized microbial CP (MCP), and (3) rumen endogenous CP (ECP), calculated as MP (g/kg of DM) = $ARUP^{NRC}$ + $AMCP^{NRC}$ + AECP.

Degraded Protein Balance. Degraded protein balance (**DBP^{NRC}**), based on data from the NRC-2001 model, reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on energy (discounted TDN) available for microbial fermentation in the rumen. The DBP^{NRC} was calculated as DPB^{NRC} (g/kg of DM) = RDP^{NRC} − 1.18 × $MCP_{TDN.}$

Nutrient Supply with the PDI System

The principle of the PDI system (Verity and Geay, 1987; INRA, 1978) has been used in this study to calculate the true Protein truly Digestible in the small Intestine (PDI) value for different feed materials. The PDI content of a diet is the sum of two fractions: PDIA: the dietary protein undegraded in the rumen, but truly digestible in the small intestine and PDIM: the microbial true protein which is truly digestible in the small intestine. Each feed contributes to microbial protein synthesis both by the degradable and the available energy it supplies to the rumen microorganisms. Thus each feed is characterised by two PDIM

values. 1) PDIMN, which corresponds to the amount of microbial protein that could be synthesized in the rumen from the degraded dietary N, when energy and others nutrients are not limiting. 2) PDIME, which corresponds to the amount of microbial protein that could be synthesized from the energy available in the rumen, when degraded N and other nutrients are not limiting.

The value of each feed is given directly as the sum of PDIA and PDIM, considering separately each of the two possible situations:

PDIN = PDIA + PDIMN and PDIE = PDIA + PDIME.

The PDI values were obtained from four individual feed characteristics: 1) CP content, 2) degradability of crude protein (RDP^{PDI}) obtained from the rumen incubation procedure, 3) fermentable organic matter content (FOM) calculated from the total digestible organic matter (DOM) content after subtraction of the contents of ether extract and undegradable dietary protein in the feed and fermentation products in silage, 4) true intestinal digestibility (TId) of rumen undegraded dietary true protein (RUP^{PDI}).

Estimation of Microbial Protein Synthesis in the Rumen based on Available Energy or on Ruminally Degraded Protein and Truly Absorbable Rumen Synthesized Microbial Protein in the Small Intestine. The microbial protein synthesis was predicted from FOM. Fermentable organic matter content was calculated as follows: FOM = DOM - EE - RUP^{PDI} (%CP) and PDIME was calculated as PDIME = FOM × 0.145 × 0.8 × 0.8. The factor 0.145 represents the yield of microbial protein that is assumed to be 145 g CP/kg of FOM with regard to energy substrates; the amino acid content of both microbial protein and their true digestibility in the small intestine are assumed to be constant, and equal to 0.8.

The PDIMN was calculated as PDIMN = CP × (1-1.11 (1-RDP^{PDI})) × 0.9 × 0.8 × 0.8, where 0.9 is the efficiency of conversion of degraded N to rumen microbial N and as mentioned before, the amino acid content of microbial protein and their true digestibility in the small intestine are 0.8 and 0.8, respectively. The truly absorbable rumen synthesized microbial protein in the small intestine (MPS^{PDI}) was calculated as MPS^{PDI} (g/kg of DM) = CP ×RDP^{PDI}/DOM.

Estimation of Rumen Undegraded Feed Protein and Truly Absorbed Rumen Undegraded Feed Protein in the Small Intestine. The RDP^{PDI} was assessed from the disappearance of protein from nylon bags, assuming a rumen particle outflow rate equal to 0.06 h^{-1}. The PDIA was calculated as PDIA = CP × (1.11 (1- RDP^{PDI})) × TId, where the effective rumen by-pass of protein is assumed to be 1.11 × (1- RDP^{PDI}).

True intestinal digestibility of rumen undegraded dietary protein. The TId was calculated as TId (g/kg of DM) = 88.3 × 0.371 × CP -0.0037 × CP^2 – 1.07 × ADL – 0.313 × UDOM, where CP and acid detergent lignin **(ADL)** are expressed in (g/kg of DM) and **(UDOM)** represents the indigestible organic matter. [16]

Estimation of the degraded protein balance. Degraded protein balance (DPB^{PDI}) was calculated as DPB^{PDI} (g/kg of DM) = (PDIA+PDIMN) – (PDIA+PDIME).

In order to make the comparison of the microbial PDIME or PDIMN, with the DVE/OEB system and NRC- 2001 model, PDIME and PDIMN values were re-calculated as:

PDIME = FOM × 0.145, PDIMN = CP × (1-1.11 (1- RDP^{PDI})) and PDIA = CP × (1.11 (1-RDP)) × dRUP.

The standard coefficients that used to describe the digestive process were not used for the re-calculation of the PDIME and PDIMN values. The reason is that at the DVE/OEB system and the NRC-2001 model, the efficiency of conversion of degraded N to rumen microbial N, the content of amino acids and their digestibility are not taken into account. Also, PDIA was re-calculated by using the value of dRUP, as used during the calculations for the other two models.

Feed Milk Value Determined Based on Metabolic Characteristics of Protein

Based on metabolic characteristics of protein from the DVE, NRC and PDI model, the feed milk value were determined. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67

and protein composition in milk is assumed 33 g protein per 1000 g milk.

Statistical Analysis

Statistical analyses were performed using the MIXED procedure of SAS (version 9.2) SAS Institute, Inc., Cary, NC, 2008). Data were analyzed with a CRD model: $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the effect of feed sources, as a fixed effect (different samples as replications), and e_{ij} was the random error associated with the observation ij. Proc Univarite with Normal and Plot options was used to check residual assumption of CRD analysis.

Comparisons among the models were performed using the MIXED procedure of SAS 9.2 and analysed with the multi-comparison procedure. Regression analysis among the models was performed using the REG procedure of SAS. Proc Univarite with Normal and Plot options was used to check residual assumption of regression analysis.

The significance of differences between means was assessed using Tukey's test. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

Results

Protein Supply to Dairy Cattle Using the DVE/OEB System

The effects of processing method and canola variety on protein supply to dairy cows obtained by using the DVE/OEB system are presented in Table 1. Comparing CM_Y and CM_B no significant differences were observed for FOM, MCP_{FOM} and $MCP_{RDP}{}^{DVE}$. Consequently, $AMCP^{DVE}$ was not different between the two canola varieties used in this study. Canola presscake was lower ($P < 0.05$) compared to both canola meals (435 vs. 556 mean value) and (65 vs. 84 mean value) for FOM and $MCP_{FOM,}$ respectively. For CM_Y endogenous protein loss in the small intestine was lower ($P < 0.001$) than CM_B and CPC_B. The DVE value for CM_Y was nearly two times higher ($P < 0.05$) than that for CM_B. Comparing the two varieties of canola meal (CM_Y and CM_B) with CPC_B, there were significant differences in truly digested protein in small intestine, and CPC_B had lower ($P < 0.05$) DVE value (Table 1). The OEB values for all treatments were determined to be positive with significant difference between the CM_Y and CM_B. The OEB value was found to be higher ($P < 0.05$) for CM_B than for CM_Y (104 vs. 84 g/kg DM)

Protein Supply to Dairy Cattle Using the NRC-2001 Model

Table 2 shows the results of using the NRC dairy model [7] to predict the potential nutrient supply of total metabolisable protein to dairy cattle from canola meal and presscake as affected by processing method. The $ARUP^{NRC}$ was significantly different between the two varieties of canola meal and the higher value was obtained for CM_Y. Moreover, CM_B was significantly lower than CPC_B, in MCP_{TDN} and therefore lower in $AMCP^{NRC}$. The CPC_B was significantly lower in RUP^{NRC} which resulted in a lower ($P < 0.05$) value for the absorption of the $ARUP^{NRC}$ compare to canola meal (104 vs. 183 mean values). Total metabolisable protein calculated from $AMCP^{NRC}$, AECP and $ARUP^{NRC}$, was higher ($P < 0.05$) for CM_Y than CM_B and CPC_B (Table 2). No significant differences were detected in DPB^{NRC} among the feedstuffs used in this study.

Protein Supply to Dairy Cattle Using the PDI system

Prediction of the potential nutrient supply to dairy cattle from canola co-products, as affected by processing method, using the PDI system is shown in Table 3. Fermentable organic matter was higher ($P < 0.05$) for both canola meals than CPC_B, which resulted in a higher amount of PDIME. So, PDIME value was 50, 46 and 39 for CM_Y, CM_B and CPC_B, respectively ($P < 0.05$). The value of microbial protein synthesis in the rumen was significantly lower for CM_Y than the one for CM_B (Table 3). In relation with its higher ($P < 0.05$) RUP^{PDI} the CM_Y had also a higher ($P < 0.05$) amount of PDIA,

compared to the other treatments. The highest ($P < 0.05$) value of digestible protein in the small intestine was obtained for CM_Y compared to other treatments. Also, the balance between microbial protein synthesis, from available rumen degradable protein and potential energy in the rumen (DPB), was lower for CM_Y compared to CM_B ($P < 0.05$) and CPC_B ($P > 0.05$).

Feed Milk Value Determined Based on Metabolic Characteristics of Protein

The feed milk value (kg milk yield per kg feed) of brown canola meal (CM: *B. Napus*) and yellow canola meal (CM: *B. Juncea*) in comparison with brown canola presscake (CPC: *B. Napus*) based on metabolic characteristics of protein predicted by DVE, NRC and PDI system are shown in Table 4. Based one MP value from DVE system, the results show that CM-Yellow (*B. Juncea)* had higher ($P < 0.05$) feed milk value than CM-Brown (*B. Napus*) and CPC-Brown (*B. Napus*) (6.3 kg milk vs. 3.9 kg milk and 2.6 kg milk, respectively). Similar trends are found for NRC and PDI prediction with CM-Yellow had significantly higher feed milk value than CM-Brown and CPC-Brown.

Comparisons among DVE/OEB System, NRC-2001 Model and PDI System in Prediction of Protein Supply to Dairy Cows

The averages of the predicted values for CM_Y, CM_B and CPC_B, modelled according to the DVE/OEB system, the NRC-2001 model and the PDI system, as well as the comparison among those models are presented in Table 5.

Comparison between DVE/OEB System and NRC-2001 Model in the Prediction of Protein Supply to Dairy Cows.

Using the DVE/OEB system, the mean supply of endogenous protein was higher ($P < 0.05$) by 14 g/kg of DM than the value predicted with NRC-2001 model. Moreover, even though the MCP_{TDN} and $AMCP^{NRC}$ values were 12.7% and 13.2%, respectively, greater in the NRC-2001 model than DVE/OEB system, differences between these values did not reach a significant level ($P > 0.05$) (Table 6).

Linear regression equations of the main average predicted nutritional values between the NRC-2001 model and the DVE/OEB system with the different types of canola meal are presented in Table 6. The results indicated that not all the regression equations were significant ($P > 0.05$); however a high proportion of the variability in truly absorbed rumen-undegraded feed protein in the small intestine ($R^2 = 1.00$) and predicted total metabolisable protein ($R^2 = 0.97$) according to the DVE/OEB system can be accounted for in the equivalent parameters predicted by the NRC-2001 model.

Comparison between PDI System and DVE/OEB system in the Prediction of Protein Supply to Dairy Cows.

The results indicated that the predicted values from the PDI system were 19% lower ($P < 0.05$) in the truly absorbed rumen-synthesized microbial protein than the predicted values from the DVE/OEB system (Table 5). No significant differences were detected in terms of truly absorbed rumen-undegraded feed protein; microbial protein supply based on available energy and degraded protein balance between the two models.

Linear regression of the predicted nutritional values between the PDI and the DVE/OEB system are presented in Table 7. All the regression equations were significant ($P < 0.05$) and all the parameters predicted by the PDI system can be accounted for by the equivalent parameters predicted by the DVE/OEB system.

Comparison between the NRC-2001 Model and PDI System and in Prediction of Protein Supply to Dairy Cows.

The comparison between the NRC-2001 model and PDI system is presented in Table 5. The results showed that using the PDI system, the overall mean for microbial protein supply, based on energy and truly absorbed rumen-synthesized microbial protein, were lower (-17 and -14 g/kg of DM, respectively) than the same values predicted by the NRC-2001 model.

Linear regression of the main average predicted nutritional values between the PDI system and the NRC-2001 model are presented in Table 8. The regression equations were significant ($P < 0.05$) for the truly absorbed rumen-synthesised microbial protein ($R^2 = 0.71$) and the truly absorbed rumen-undegraded feed protein ($R^2 = 1.00$) predicted according to the PDI system.

Discussion

Metabolic Characteristics of Protein in Canola Co-Products

Comparing three-types of canola co-products, there were significant differences in the truly absorbed protein supply, protein degraded balance and feed milk value among the different types of canola co-products. Yellow-seeded canola meal had significantly higher intestinal digestibility of rumen undegraded crude, total metabolizable protein and feed milk value, but lower degraded protein balance than brown-seeded canola meal and presscake (Tables 1-3). All canola co-products had higher metabolizable protein level than barley, oat, triticale and wheat[22,23] This results also showed that feed milk values are much higher than cereal grain (Table 4). Low protein degraded balance indicated lower potential N loss than cereal grains. All these results indicate that canola co-products, particularly yellow-type canola co-products are excellent metabolizable protein sources.

Based on an increased knowledge gained concerning the ruminants' N metabolism, different evaluation systems have been developed in order to quantitatively predict protein nutrient supply to dairy cows. This study provides information on the prediction of protein supply to dairy cows by employing and comparing three different evaluation systems.

Prediction of Endogenous Protein

A notable difference is the concept and calculation of endogenous protein in the digestive process. In the DVE/OEB system, the truly digested and absorbed protein in the small intestine requires a correction for endogenous protein losses, [11] which are affected by undigested dry matter. According to the DVE/OEB system, 75 g of absorbed protein per kg undigested DM in fecal excretion is required to compensate for the endogenous losses. In the NRC-2001 model, calculation of the metabolisable protein (MP) value considers rumen endogenous protein (AECP) passed on to the small intestine and contributes to the total metabolisable protein value. Also the rumen endogenous protein is associated with dry matter content. Although the endogenous protein losses in the small intestine are taken into account by the NRC-2001 model, it´s value is added to requirements rather than subtracted from supply.

In our study the DVE value predicted by the DVE/OEB system was about 2.5% lower than the value obtained by using NRC-2001 model. The same trend was in forages (alfalfa and timothy) [17] in which the amounts of total absorbable protein supply to small intestine predicted by using DVE/OEB system (DVE values) were 15% lower than predictions by the NRC-2001 model (MP values). In agreement with these results, Heendeniya et al. [18] predicted that DVE values were lower than MP values for canola meal.

Furthermore, it should be mentioned that in the DVE/OEB system the endogenous protein (ENDP) is considered as a loss. Thus, a comparatively higher ENDP value was estimated from DVE/OEB model for canola meal and presscake compared to AECP.

In contrast with the previous mentioned models, the PDI system does not consider the endogenous protein for the calculation of the truly absorbed protein in the small intestine. Therefore, no comparison was made between PDI and the other two evaluation systems, not only for endogenous but for total metabolisable protein as well.

Prediction of Truly Absorbed Rumen-Undegraded Feed Protein

In all the three models compared, the truly absorbed rumen-undegraded feed protein in the small intestine was calculated as the product of the rumen-undegraded feed protein and the digestibility of feed protein in the intestine. However, the prediction of rumen-undegraded feed protein differs among the models; while the DVE/OEB and the PDI system use a coefficient (1.11) to correct the in situ degradation

data on in vivo results, no correction factor is used by the NRC-2001 model.

Prediction of Microbial Protein Synthesis in the Rumen Based on Available Energy

The prediction of the potential microbial protein synthesized in the rumen from all three models used in this study was based on available energy. The DVE/OEB system, as well as the PDI system use rumen fermented OM as the energy base to predict microbial protein. However, the NRC-2001 model uses available TDN as its energy base. Furthermore, each model uses different factor parameters to calculate microbial protein synthesized in the rumen.

Another difference among the evaluation systems compared concerns the microbial protein synthesis. Specifically, DVE/OEB system assumes 150 g of microbial protein to be synthesized per kg fermented OM, PDI system assumes 145 g of microbial protein to be synthesized with regard to energy substrates and NRC-2001 model assumes 130 g. of microbial protein CP is to be synthesized per kg TDN. Moreover, the amount of truly absorbable rumen-synthesized microbial protein was calculated differently among the models. In the DVE/OEB system, the amount of truly absorbed rumen synthesized microbial protein in the small intestine was estimated as: $0.85 \times 0.75 \times MCP_{FOM}$ as the system assumes that true digestibility of microbial protein is 85%,[19] and 75% of microbial N is present in amino acids; the remaining is N in nucleic acids. In the NRC-2001 model, digestibility and true protein of ruminally synthesized microbial CP are assumed to be 80%; therefore, the amount of truly absorbed rumen synthesized microbial protein in the small intestine was estimated as: $0.80 \times 0.80 \times MCP$. Although the individual coefficients differ, the net result is essentially the same between the two models (0.85×0.75) vs. (0.80×0.80). In our study no significant difference was detected in terms of the microbial protein supply based on available energy. This is in contrast with Yu et al. [8] who found for different concentrate feeds (barley, beans, lupins, soybeans) that the overall average microbial protein supply, based on available energy, was 10% lower than that predicted by the NRC-2001 model. The reason for such difference may be due to feed types.

In the PDI system, the PDIME was estimated as: CP × RDP / DOM. This difference in quantifying calculation as well as the concept on the microbial protein supply based on available energy, resulted in a lower PDIME value compared with MCP_{TDN} by almost 19%.

Prediction of Microbial Protein Supply Based on Ruminally Degraded Feed Protein

The concept and calculation for the prediction of microbial protein supply based on ruminally degraded feed protein among models is different. In the DVE/OEB system, [11] is assumed that 100% ruminally degraded feed protein could be potentially converted to microbial protein if enough energy is provided. However, in the NRC-2001 model, is assumed that only 85% of ruminally degraded feed protein could be potentially converted to microbial protein. In the PDI system, it is assumed that 90% of the rumen degraded protein is converted to microbial protein due to the reason that some unavoidable losses such as unusable N fraction or rumen outflow may occur. [20,21]

A comparison in the prediction of nutrient supply to dairy cows from forages between the DVE/OEB system and NRC-2001 model has been carried out by Yu et al. [17] In that study it was found that AMCP and ARUP values derived from DVE/OEB system, were consistently higher that those derived from the NRC-2001 model, for both alfalfa and timothy samples. However, in our study, only the $ARUP^{DVE}$ value was observed to be numerically higher than that predicted by NRC-2001 model. In agreement with our results, Heendeniya et al. [18] found that the AMCP and ARUP values predicted for canola meal using the DVE/OEB system were lower than those of the NRC-2001 model. Nevertheless, at the same study they noted that the opposite results were found for soy meal.

Prediction of Degraded Protein Balance

The Degraded Protein Balance (DPB) shows the (im)-balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen. When the DPB in a ration is positive, it indicates the potential N-loss from the rumen and when negative, microbial protein synthesis is predicted to be impaired because of a potential shortage of N in the rumen. The optimum degraded protein balance value in a ration is, therefore, zero or slightly higher.[11] The

predicted protein balances (DPB) by the three models showed that all the feedstuffs exhibited positive DPB values. This indicates that availability of feed protein exceeds the availability of energy (extracted during rumen fermentation) for microbial protein synthesis in all different types of canola samples, which results in a potential nitrogen loss in the rumen. [11]

Generally, based on the findings of the current study and published results by others [18,22,23] it appears that, these three models could interact with different factors as feed type, processing method or variety. Therefore, standardization of sample processing and analytical procedures and grouping the feeds into categories based on chemical and physical characteristics may increase predictability and accuracy of data extrapolation from one model to another.

Conclusion

Comparing three-types of canola co-products, there were significant differences in the truly absorbed protein supply, protein degraded balance and feed milk value among the different types of canola meal. Yellow-seeded canola meal had significantly higher intestinal digestibility of rumen undegraded crude, total metabolizable protein and feed milk value, but lower degraded protein balance than brown-seeded canola meal and presscake. Comparing the DVE/OEB with the NRC-2001 model, not all the regression equations were significant; however, a high proportion of the variability in truly absorbed rumen-undegraded feed protein in the small intestine and the total metabolisable protein predicted by DVE/OEB system was found that can be accounted for the equivalent parameters predicted by the NRC-2001 model. The results show that the truly absorbed rumen-synthesized microbial protein values predicted from PDI system were 19% lower than those predicted from the DVE/OEB system. All the parameters predicted by the PDI system can be accounted for by the equivalent parameters predicted by the DVE/OEB system. When comparing PDI system and NRC-2001 model, the overall means for microbial protein supply based on energy and truly absorbed rumen-synthesized microbial protein were found to be lower than those predicted by the NRC-2001 model. By using all the protein evaluation systems compared in this study it is possible to predict the potential nutrient supply to the animal from feedstuffs as affected by processing. Due to the fact that the results reported here were outputs from models with inputs based on in vitro and in situ studies, the challenge is to apply the prediction and evaluate them in animal experiments. However, the number of such studies in this area available to challenge the model is extremely limited.

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Table 1. Predicted values of potential nutrient supply to dairy cattle from brown canola meal (CM: *B. Napus*) and yellow canola meal (CM: *B. Juncea*) in comparison with brown canola presscake (CPC: *B. Napus*) using the Dutch DVE/OEB system

	Type of Canola products					Contrast, *P* value
Item (g/kg of DM)	CM-Yellow *B. Juncea*	CM-Brown *B. Napus*	CPC-Brown *B. Napus*	SEM[1]	*P* value	CM vs. CPC
Absorbable microbial protein synthesis in the rumen ($AMCP^{DVE}$)[2]						
FOM	579.8[a]	533.0[a]	434.8[b]	14.18	0.012	0.006
MCP_{FOM}	87.0[a]	80.0[a]	65.2[b]	2.13	0.012	0.006
$MCP_{RDP}{}^{DVE}$	171.1[a]	184.1[a]	167.2[a]	3.88	0.110	0.117
$AMCP^{DVE}$	55.5[a]	51.0[a]	41.6[b]	1.36	0.012	0.006
Endogenous protein in the small intestine (ENDP)[3]						
ENDP	12.9[b]	20.2[a]	20.5[a]	0.27	0.001	0.001
Truly absorbable rumen un-degraded protein in small intestine ($ARUP^{DVE}$)[4]						
RUP^{DVE}	250.2[a]	185.3[b]	167.2[b]	3.94	0.001	0.002
dRUP						0.005
	903.8a	749.1b	601.4c	24.40	0.007	
$ARUP^{DVE}$	269.7[a]	161.7[b]	107.6[b]	9.44	0.003	0.003
Total truly digested protein in small intestine (DVE value)[5]						
DVE	312.2[a]	192.5[b]	128.6[c]	10.79	0.003	0.003
Degraded protein balance (OEB value)[6]						
DPB^{DVE}	84.2[b]	104.1[a]	102.0[a]	1.76	0.007	0.035

[a - c] Means within a row with different superscripts differ ($P < 0.05$).

[1]SEM = Standard error of mean.

[2]FOM = organic matter fermented in the rumen; MCP_{FOM} = microbial protein synthesized in the rumen based on available energy; $MCP_{RDP}{}^{DVE}$ = microbial protein synthesized in the rumen based on rumen degraded feed crude protein; AMCP = truly absorbed rumen synthesized microbial protein in the small intestine.

[3]ENDP = endogenous protein losses in the digestive tract.

[4]RUP^{DVE} = ruminally undegraded feed CP, calculated according the formula in DVE/OEB system; $ARUP^{DVE}$ = truly absorbed bypass feed protein in the small intestine.

[5]DVE = truly absorbed protein in the small intestine contributed by 1) feed protein escaping rumen degradation (RUP^{DVE}), 2) microbial protein synthesized in the rumen (MCP_{FOM}) and 3) a correction for endogenous protein losses in the digestive tract (ENDP).

[6]DPB^{DVE} = reflects the difference between the potential microbial protein synthesis based on rumen degraded feed crude protein (CP) and that based on energy (rumen fermented OM) available for microbial fermentation in the rumen.

Table 2. Predicted values of potential nutrient supply to dairy cattle from brown canola meal (CM: *B. Napus*) and yellow canola meal (CM: *B. Juncea*) in comparison with brown canola presscake (CPC: *B. Juncea*) using the NRC-2001 model

	Type of Canola products					Contrast, *P* value
Item (g/kg of DM)	CM-Yellow *B. Napus*	CM-Brown *B. Juncea*	CPC-Brown *B. Napus*	SEM[1]	*P* value	CM vs. CPC
Absorbable microbial protein synthesis in the rumen ($AMCP^{NRC}$)[2]						
$MCP_{TDN}{}^{NRC}$	89.0^{ab}	77.8^{b}	94.9^{a}	2.24	0.027	0.024
$MCP_{RDP}{}^{NRC}$	158.8^{a}	166.5^{a}	163.6^{a}	1.43	0.071	0.627
$AMCP^{NRC}$	56.9^{ab}	49.8^{b}	60.8^{a}	1.43	0.027	0.025
Absorbable endogenous true protein in the small intestine (AECP)[3]						
ECP	10.6^{b}	10.5^{b}	11.2^{a}	0.04	0.002	0.001
AECP	4.2^{b}	4.2^{b}	4.5^{a}	0.02	0.002	0.001
Truly absorbable rumen un-degraded protein in small intestine ($ARUP^{NRC}$)[4]						
RUP^{NRC}	250.2^{a}	185.3^{b}	167.2^{b}	3.94	0.001	0.002
dRUP	903.8^{a}	749.1^{b}	601.4^{c}	24.40	0.007	0.005
$ARUP^{NRC}$	226.2^{a}	138.8^{b}	103.5^{c}	5.34	0.001	0.001
Total metabolisable protein (MP)[5]						
MP	287.3^{a}	192.8^{b}	168.8^{b}	4.69	0.001	0.001
Degraded protein balance (DPB^{NRC})[6]						
DPB^{NRC}	81.9^{a}	104.0^{a}	80.4^{a}	4.21	0.048	0.094

$^{a-c}$ Means within a row with different superscripts differ ($P < 0.05$).

[1]SEM = Standard error of mean.

[2]$MCP_{TDN}{}^{NRC}$ = microbial protein synthesized in the rumen based on available energy (discounted TDN); $MCP_{RDP}{}^{NRC}$ = microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; $AMCP^{NRC}$ = truly absorbed rumen synthesized microbial protein in the small intestine.

[3]ECP = rumen endogenous crude protein (CP); AECP = truly absorbed endogenous protein in the small intestine.

[4]RUP^{NRC} = ruminally undegraded feed CP, calculated according the formula in NRC-2001 dairy model; dRUP = intestinal digestibility of rumen undegraded crude protein, estimated according to Calsamiglia and Stern, 1995; $ARUP^{NRC}$ = truly absorbed rumen undegraded feed protein in the small intestine.

[5]MP = metabolizable protein (true protein that is digested postruminally and the component amino acid absorbed by the intestine) contributed by 1) ruminally undegraded feed CP, 2) ruminally synthesized microbial CP and 3) endogenous CP.

[6]DPB^{NRC} = reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

Table 3. Predicted values of potential nutrient supply to dairy cattle from brown canola meal (CM: *B. Napus*) and yellow canola meal (CM: *B. Juncea*) in comparison with brown canola presscake (CPC: *B. Napus*) using the French PDI system

	Type of Canola products					Contrast, *P* value
Item (g/kg of DM)	CM-Yellow *B. Juncea*	CM-Brown *B. Napus*	CPC-Brown *B. Napus*	SEM[1]	*P* value	CM vs. CPC
Absorbable microbial protein synthesis in the rumen (MPS^{PDI})[2]						
DOM	824.6[a]	731.6[b]	731.8[b]	3.14	<0.001	0.001
FOM	533.1[a]	496.9[a]	424.0[b]	11.30	0.014	0.007
PDIME	49.5[a]	46.1[a]	39.3[b]	1.05	0.014	0.007
PDIMN	98.6[a]	106.0[a]	96.3[a]	2.23	0.107	0.117
MPS^{PDI}	38.9[b]	44.9[a]	40.4[ab]	0.97	0.046	0.293
Truly absorbable rumen un-degraded protein in small intestine (PDIA)[3]						
RUP^{PDI}	250.2[a]	185.3[b]	167.2[b]	3.94	0.001	0.002
TId	883.6[a]	779.7[b]	768.8[b]	4.41	0.001	0.001
PDIA	263.6[a]	168.2[b]	137.2[b]	6.61	0.002	0.002
Degraded protein balance (DPB^{PDI})[4]						
PDIN (PDIN=PDIA+PDIMN)	362.2[a]	274.2[b]	233.5[b]	8.65	0.004	0.004
PDIE (PDIE=PDIA+PDIME)	313.1[a]	214.3[b]	176.6[b]	7.20	0.002	0.002
DPB^{PDI} (PDIN-PDIE)	49.1[b]	59.9[a]	57.0[ab]	1.52	0.038	0.278

[a - c] Means within a row with different superscripts differ ($P < 0.05$).

[1]SEM = Standard error of mean.

[2]DOM = digestible organic matter; FOM = fermentable organic matter in the rumen calculated from the DOM; PDIME = amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded nitrogen (N) is not limiting; PDIMN = of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; MPS = truly absorbed rumen synthesized microbial protein in the small intestine.

[3]RUP^{PDI} = ruminally undegraded feed crude protein (CP); TId = true digestibility in the small intestine of the undegraded dietary true protein; PDIA = dietary protein undegraded in the rumen, but truly digestible in the small intestine.

[4]PDIN = digestible proteins in the small intestine where N is the limiting factor for rumen microbial activity; PDIE = digestible proteins in the small intestine where energy is the limiting factor for rumen microbial activity; DPB^{PDI} = balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen.

Table 4. Feed milk value of brown canola meal (CM: *B. Napus*) and yellow canola meal (CM: *B. Juncea*) in comparison with brown canola presscake (CPC: *B. Napus*) based on metabolic characteristics of protein predicted by DVE, NRC and PDI system

	Canola meal treatment					Contrast, *P* value
Item (kg milk per kg feed)	CM-Yellow *B. Juncea*	CM-Brown *B. Napus*	CPC-Brown *B. Napus*	SEM[1]	*P* value	CM vs. CPC
Using DVE system Feed milk value	6.34[a]	3.91[b]	2.61[c]	0.22	0.003	0.003
Using NRC system Feed milk value	5.83[a]	3.91[b]	3.43[b]	0.10	0.001	0.001
Using PDI system Feed milk value	7.35[a]	5.57[b]	4.74[b]	0.18	0.004	0.004

[a - c] Means within a row with different superscripts differ ($P < 0.05$).

[1]SEM = Standard error of mean.

[2]The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (source NRC, 2001) and protein composition in milk is assumed 33 g protein per 1000 g milk.

Table 5. Comparison of the DVE/OEB system, the NRC-2001 model and the PDI system in the prediction of protein supply to dairy cows from the feedstuffs canola meal and canola presscake

Item (g/kg of DM)	Mean			SEM[1]	*P* value	Contrast, *P* value			
	DVE/ OEB	NRC 2001	PDI			DVE vs NRC	DVE vs. PDI	NRC vs. PDI	NRC vs. DVE+PDI
Compared microbial protein supply based on available energy[2]									
MCP_{FOM} vs MCP_{TDN} vs PDIME	77.4[ab]	87.2[a]	70.3[b]	3.54	0.015	0.068	0.176	0.004	0.007
Compared microbial protein supply based on ruminally degraded feed protein[3]									
MCP_{RDP}^{DVE} vs MCP_{RDP}^{NRC} vs PDIMN	174.1[a]	163.0[a]	174.1[a]	3.12	0.034	0.023	1.000	0.023	0.010
Compared truly absorbed rumen-synthesised microbial protein[4]									
$AMCP^{DVE}$ vs $AMCP^{NRC}$ vs MPS^{PDI}	49.3[a]	55.8[a]	41.4[b]	2.09	<0.001	0.044	0.017	<0.001	0.001
Compared truly absorbed rumen-undegraded feed protein[5]									
$ARUP^{DVE}$ vs $ARUP^{NRC}$ vs PDIA	179.6[a]	156.2[a]	180.6[a]	28.03	0.784	0.562	0.981	0.546	0.496
Compared endogenous protein[6]									
ENDP vs AECP	17.9[a]	4.3[b]	-	1.11	<0.001	<0.001	-	-	-
Compared total metabolisable protein[7] (truly absorbed protein)									
DVE vs MP	211.1[a]	216.3[a]	-	29.23	0.9025	0.9025	-	-	-
Compared degraded protein balance[8]									
DPB^{OEB} vs DPB^{NRC} vs DPB^{PDI}	96.8[a]	88.8[a]	103.9[a]	4.34	0.078	0.212	0.265	0.026	0.046

Table 6. Regression equations for prediction of protein supply from DVE/OEB system based on values from NRC-2001 model for feedstuffs canola meal and canola presscake

	Linear regression equation			
Item (g/kg of DM)	Equation Y = a (± SE) + b (± SE) × x	R^2	*P* value	RSD[1]
Microbial protein supply based on available energy[2]				
MCP_{FOM} vs MCP_{TDN}	MCP_{FOM} = 137.93 (± 45.56) - 0.69 (± 0.52) × MCP_{TDN}	0.31	0.253	9.49
Microbial protein supply based on ruminally degraded feed protein[3]				
$MCP_{RDP}{}^{DVE}$ vs $MCP_{RDP}{}^{NRC}$	$MCP_{RDP}{}^{DVE}$ = -85.54 (± 142.34) + 1.59 (± 0.87) × $MCP_{RDP}{}^{NRC}$	0.45	0.142	7.40
Predicted truly absorbed rumen-synthesised microbial protein[4]				
$AMCP^{DVE}$ vs $AMCP^{NRC}$	$AMCP^{DVE}$ = 87.95 (± 29.05) - 0.69 (± 0.52) $AMCP^{NRC}$	0.31	0.253	6.05
Predicted truly absorbed rumen-undegraded feed protein[5]				
$ARUP^{DVE}$ vs $ARUP^{NRC}$	$ARUP^{DVE}$ = - 24.93 (± 7.28) +1.31 (± 0.0044) × $ARUP^{NRC}$	1.00	<0.0001	5.62
Predicted endogenous protein[6]				
ENDP vs AECP	ENDP = -37.44 (± 52.99) + 12.84 (± 12.30) × AECP	0.21	0.355	3.80
Predicted total metabolisable protein[7] (truly absorbed protein)				
DVE vs MP	DVE = - 107.47 (± 28.77) + 1.47 (± 0.13) × MP	0.97	0.0003	16.30
Predicted degraded protein balance[8]				
DPB^{OEB} vs DPB^{NRC}	DPB^{OEB} = 57.82 (± 29.25) + 0.44 (± 0.33) × DPB^{NRC}	0.31	0.250	9.28

[1]Residual standard deviation.

Table 7. Regression equations for prediction of protein supply from PDI system based on values from DVE/OEB system for feedstuffs canola meal and canola presscake

	Linear regression equation			
Item (g/kg of DM)	Equation y = a (±SE) + b (±SE) × x	R^2	*P* value	RSD[1]
Predicted microbial protein supply based on available energy[2]				
PDIME vs MCP_{FOM}	PDIME = 14.52 (± 4.10) + 0.72 (± 0.05) × MCP_{FOM}	0.98	0.0002	1.20
Predicted microbial protein supply based on ruminally degraded feed protein[3]				
PDIMN vs $MCP_{RDP}{}^{DVE}$	PDIMN= (1.00± 0) × $MCP_{RDP}{}^{DVE}$	1.00	< 0.0001	0
Predicted truly absorbed rumen-synthesised microbial protein[4]				
MPS^{PDI} vs $AMCP^{DVE}$	MPS^{PDI}= 40.48 (± 11.41) + 0.02 (± 0.23) × $AMCP^{DVE}$	0.93	0.002	3.34
Predicted truly absorbed rumen-undegraded feed protein[5]				
PDIA vs $ARUP^{DVE}$	PDIA= 4.16 (± 2.58) + 0.98 (± 0.01) × $ARUP^{DVE}$	1.00	< 0.0001	2.24
Predicted degraded protein balance[6]				
(PDIN-PDIE) DPB^{PDI} vs DPB^{OEB}	DPB^{PDI} = 22.55 (± 12.62) + 0.84 (± 0.13) × DPB^{OEB}	0.91	0.003	2.90

[1]Residual standard deviation.

[2]PDIME = amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded nitrogen (N) is not limiting; MCPFOM = microbial protein synthesized in the rumen based on available energy.

[3]PDIMN = amount of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; MCP_{RDP} = microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein.

Table 8. Regression equations for prediction of protein supply from PDI system based on values from NRC-2001 model for feedstuffs canola meal and canola presscake

	Linear regression equation			
Item (g/kg of DM)	Equation y = a (±SE) + b (±SE) × x	R^2	*P* value	RSD[1]
Predicted microbial protein supply based on available energy[2]				
PDIME vs MCP_{TDN}	PDIME = 113.11 (± 33.57) - 0.49 (± 0.38) × MCP_{TDN}	0.29	0.269	6.99
Predicted microbial protein supply based on ruminally degraded feed protein[3]				
PDIMN vs MCP_{RDP}^{NRC}	PDIMN = -85.54 (±142.34) + 1.59(± 0.87)×MCP_{RDP}^{NRC}	0.45	0.142	7.40
Predicted truly absorbed rumen-synthesised microbial protein[4]				
MPS^{PDI} vs $AMCP^{NRC}$	MPS^{PDI} = 68.37 (± 8.66) - 0.48 (± 0.15) ×$AMCP^{NRC}$	0.71	0.035	1.80
Predicted truly absorbed rumen-undegraded feed protein[5]				
PDIA vs $ARUP^{NRC}$	PDIA = -20.66 (± 4.76) + 1.29 (± 0.03) × $ARUP^{NRC}$	1.00	<0.0001	3.67
Predicted degraded protein balance[6]				
(PDIN-PDIE) DPB^{PDI} vs DPB^{NRC}	DPB^{PDI} = 58.49 (± 20.89) + 0.51 (± 0.23) × DPB^{NRC}	0.55	0.093	6.63

[1]Residual standard deviation.

[2]PDIME = amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded nitrogen (N) is not limiting; MCP_{TDN} = microbial protein synthesized in the rumen based on available energy (discounted TDN).

[3]PDIMN = amount of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; MCP_{RDP} = microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; AMCP = truly absorbed rumen synthesized microbial protein in the small intestine.

[4]MPS^{PDI} = truly absorbed rumen synthesized microbial protein in the small intestine; $AMCP^{NRC}$ = truly absorbed rumen synthesized microbial protein in the small intestine.

[5]PDIA = dietary protein undegraded in the rumen, but truly digestible in the small intestine; $ARUP^{NRC}$ = truly absorbed rumen undegraded feed protein in the small intestine.

[6]DPB^{PDI} = balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen; DPB^{NRC} = reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

Project 3: REVEAL PROTEIN MOLECULAR STRUCTURES OF CANOLA MEAL AND PRESSCAKE, AS AFFECTED BY HEAT PROCESSING METHODS, IN RELATIONSHIP WITH THEIR PROTEIN DIGESTIVE BEHAVIOUR AND UTILIZATION FOR DAIRY CATTLE (COMPLETED) *

A version of this project has been published: Katerina Theodoridou and Peiqiang Yu. 2013. Application potential of ATR-FT/IR molecular spectroscopy in animal nutrition: Reveal protein molecular structures of canola meal and presscake, as affected by heat processing methods, in relationship with their protein digestive behavior and utilization for dairy cattle. Journal of Agricultural and Food Chemistry (USA). 61: 5449−5458 (DOI: 10.1021/jf400301y) (*as supervisor, PI, corresponding author).

Project Details

Abstract:

Protein quality relies not only on total protein but also on protein inherent structures. The most commonly occurring protein secondary structures (α-helix and β-sheet) may influence protein quality, nutrient utilization and digestive behavior. The objectives of this study were to reveal the protein molecular structures of canola meal (yellow and brown) and presscake as affected by the heat processing methods and to investigate the relationship between structure changes and protein rumen degradations kinetics, estimated protein intestinal digestibility, degraded protein balance and metabolisable protein. The protein molecular structures were identified using FT/IR-ATR molecular spectroscopy. The results showed that β-sheet value was higher for the yellow canola meal compared to the brown one. Processing conditions resulted in a higher value for α-helix and β-sheet for brown-seeded canola presscake compared to brown-seeded canola meal. The multivariate molecular spectral analyses (PCA, CLA) showed that there were significant molecular structural differences in the protein amide I and II finger print region (*ca* 1700-1480 cm^{-1}) among the brown canola meal and presscake. These differences were indicated by the form of separate class (PCA) and group of separate ellipse (CLA) between the feedstuffs. The *in situ* degradation parameters, the amide I, amide II and α-helix to β-sheet ratio (R_a_β) had positive correlations with the degradable fraction and the degradation rate. For the prediction of protein supply to dairy cattle, the amide I and the amide II were correlated with the truly absorbed rumen synthesized microbial protein in the small intestine ($AMCP^{NRC}$) whereas the truly absorbed endogenous protein in the small intestine was correlated with the amide I to amide II ratio. Modelling results showed that α-helix was positively correlated with both the truly absorbed rumen synthesized microbial protein in the small intestine using the Dutch DVE/OEB system ($AMCP^{DVE}$) and $AMCP^{NRC}$ and the R_a_β negatively correlated with the truly absorbed bypass feed protein in the small intestine based on the DVE/OEB system. For protein profiles, the R_a_β was a better predictor for crude protein (79%) and for neutral detergent insoluble crude protein (68%) and the only significant predictor for the degradation rate, degradable fraction, protein ruminal effective and rumen undegradable protein. In conclusion, ATR-FT/IR molecular spectroscopy could be used to characterize feed structures at molecular level rapidly and also used as a potential predictor to feed functionality, digestive behaviour and nutrient utilization of canola feed.

Keywords: Canola, Protein secondary structure, Molecular spectroscopy, α-helix, protein nutritive value, Processing condition

Introduction

Heat treatment of animal feed has been employed to improve the utilization and the availability of its protein[1] and inactivate any anti-nutrition factors[2] by reducing fermentation and metabolism in the rumen. This results in an increase of the amounts of protein entering the small intestine for further absorption and digestion.[1] The effectiveness of heat processing as a tool for optimize feed protein value, has been well documented in the literature and several mechanisms of altering the degradation and digestive behavior of protein have been proposed for the findings. Generally any temperature change in the environment of the protein which can influence the non-covalent interactions involved in the structure may lead to an alteration of the protein structure including protein secondary structures.[3]

Protein quality relies not only on total protein and amino acid content, but also on protein inherent structures such as protein secondary structures and nutrient matrix.[4] The most commonly occurring protein secondary structures include the α-helix and the β-sheet.[5,6,7] The protein secondary structure profiles may influence protein quality, nutrient utilization, availability or digestive behavior.[8,9,10,11] So for those reasons studying the secondary structure of proteins is often of interest in order to understand its digestive behavior, nutritive quality, utilization and availability in animals and humans.[10]

However, few studies on protein structures and alteration of their inherent structures due to processing conditions, in relation to nutritive value and digestive behavior of protein exist. An approach would be the use of Fourier-transformed infrared-vibration spectroscopy (FT/IR), a well-established experimental technique for studying the secondary structural composition, stability and conformational changes (effects of temperature, pH and pressure).[12,13,14] This approach to determine protein value by revealing inherent molecular structural chemical differences of protein of the canola feed will provide basic information that could be useful for the animal feed processing, the plant breeding as well as the human food industries to maintain and improve the quality of protein.

Objective

The hypothesis of this study was that heat processing methods changes the protein molecular structure in canola meal and canola presscake and that these are associated with changes in protein nutrient utilization and availability. The objectives of this study were (1) to reveal protein molecular structures of canola meal and presscake by using FT/IR-ATR molecular spectroscopy, (3) to investigate the relationship between protein molecular structures (in terms of protein α-helix and β-sheet intensity and their ratio and amide I to amide II intensity and their ratio) and protein rumen degradations kinetics (rate and extent), estimated protein intestinal digestibility, degraded protein balance and total truly absorbed protein in small intestine (metabolisable protein) and (4) to determine the most important structural features for canola which could be used as predictors of protein nutrient availability and digestive characteristics.

Material and Methods

Canola Meal and Presscake and Nutrient Analysis.

Canola meal (CM) and canola presscake (CPC) were used in this model study as a feed protein source. Two different varieties of canola: yellow-seeded (CM_Y) (*B. juncea)* and brown-seeded (CM_B) (*B. napus*) solvent-extracted canola meal were provided by the *Agriculture and Agri-Food Canada,* Lethbridge Research Center, Alberta, Canada on May 2010 and the second source by the Bunge Altona, Manitoba, Canada on October 2011. The brown-seeded (*B. napus)* canola presscake (CPC_B) was produced and obtained from Milligan Biotech (Foam Lake, Saskatchewan, Canada). In this research study were used 2 different batches for (CM_Y) (total 4 kg), 2 different batches for (CM_B) (total 4 kg) and 2 different batches for (CPC_B) (total 10 kg). The detailed methods and calculations for nutrient modeling, chemical and nutrient analysed, Cornell Net Carbohydrate and Protein System (CNCPS) protein fractionation, *in situ* rumen degradation kinetics and estimated intestinal protein digestion were reported previously by Theodoridou and Yu 2012, 2013.[15,16]

Processing Conditions of Feedstuffs

Canola seed was crushed and then was solvent-extracted in order to separate the oil from the meal. This process (pre-press solvent extraction), included the following steps:

a) Seed cleaning

Canola seed was graded according to strict grading standards (i.e. maximum moisture content, seed damage and chlorophyll level) and then was delivered to the crushing plant.

b) Seed pre-condition and flaking

The seed was pre-heated with grain dryers to approximately 35°C to prevent shattering which may occur when cold seed from storage enters the flaking unit. The cleaned seed was first flaked to physically rupture the seed coat.

c) Seed cooking

Later on, flakes were cooked / conditioned by passing them through a series of steam-heated drum or stack type cookers. At the start of cooking, the temperature was rapidly increased to 80-90°C which served to inactivate the myrosinase enzyme present in canola. The cooking cycle lasted 15-20 minutes and the temperatures normally ranged between 80° and 105°C, with an optimum of about 88°C.

d) Pressing the flake to mechanically remove a portion of the oil

The cooked canola seed flakes were then pressed in a series of screw presses or expellers. Pressing was occurred in order to remove as much oil as possible, usually 50-60% of the seed oil content, while maximizing the output of the expellers and producing a canola presscake**.**

e) Solvent extraction of the press-cake to remove the remainder of the oil

The presscake was solvent-extracted (hexane) in order to remove the remaining oil. f) Desolventizing and toasting of the meal. The solvent was removed from the marc in a desolventizer-toaster. During the desolventization-toasting process the meal was heated to 95-115°C and moisture increased to 12-18%. The total time spent in the desolventizer-toaster was approximately 30 minutes. The canola meal was then cooled and dried to approximately 12% moisture by blowing air through it. The **canola meal** was next granulated to a uniform consistency and storage.

ATR-FT/IR Data and Collection Analysis.

Molecular spectroscopic experiments were performed at the Department of Animal and Poultry Science, University of Saskatchewan. Fourier-transformed infrared-vibration spectroscopy (FT/IR) was performed using a JASCO FT/IR-ATR-4200 (Jasco Inc., Easton, MD Corp., Tokyo, Japan) with a ceramic IR light source and a deuterated L-alanine doped triglycine sulfate detector (JASCO Corporation, Tokyo, Japan) equipped with a MIRacleTM attenuated total reflectance accessory module and outfitted with a ZnSe crystal and pressure clamp (PIKE Technologies, Madison, WI, USA). Each sample was analyzed 5 times. Thirty-two scans per sample were collected in the mid-infrared range from 4.000 to 700 cm^{-1} in transmission mode at a spectral resolution of 4 cm^{-1}. The collected spectra were corrected against air as background.

Univariate Molecular Spectral Analysis of Protein Structure Profiles.

The functional spectral bands associated with protein molecular structures were identified with OMNIC 7.2 software (Spectra-Tech Inc., Madison, WI, USA) and assigned according to published studies.[17,18,19,20,21,22] Unique primary protein features found in peptide bonds (C-O, C-N and N-H) include amide I (~80% C=O and ~20% C-N stretching vibration; centered at a wavelength of *ca.* 1655 cm^{-1}) and amide II (~60% N-H bending vibration,~40% C-N stretching vibration; centered at *ca.* 1550 cm^{-1}) which

are detectable as two absorption peaks within the wavelength region from *ca.* 1720 to 1485 cm^{-1}. [23,24,20] Absorption peak heights for secondary protein structure α-helices (*ca.* 1660 cm^{-1}) and β-sheets (*ca.* 1630 cm^{-1}) are detectable in the amide I area using the second derivative function of OMNIC 7.2 (Spectra Tech, Madison, WI). The ratios of amide I and II and α-helix and β-sheet spectral intensities were calculated.

Multivariate Molecular Spectral Analysis of Protein Structure Profiles.

In order to compare the spectra of the different feedstuffs used in this study and to determine if they were some underlying structural differences multivariate spectral analyses were applied in this study. CLA results were presented as dendrograms while PCA results were plotted based on the two highest factor scores and illustrated as a function of those scores. In each comparison the eigenvector for factor 1 was plotted against that for factor 2 which accounted for over 99% of the variability in the data. These analyses were carried out using Statistica 8 software (StatSoft Inc., Tulsa, OK, USA).

Statistical Analysis.

Statistical analysis was performed using the PROC MIXED procedure statistical package of SAS. [25] The significance was declared at $P < 0.5$ and trends at $P \leq 0.10$ and the differences among treatments were evaluated using Tukey's test and means with different superscript letter groups were obtained with "pdmix800 SAS macro". [26] The FT/IR spectroscopic data were analysed using a completely randomised design model (CRD):

$$Y_{ij} = \mu + T_i + e_{ij},$$

where, Y_{ij} was an observation of the dependent variable *ij* (amide I, amide II, ratio of amide I to amide II, α-helix, β-sheet or ratio of α-helix to β-sheet), μ was the population mean for the variable; T_i was the effect of feed source (i= 1-3 CM_Y, CM_B and CPC_B), as a fixed effect, batch as replication and e_{ij} was the random error associated with the observation *ij*.

Correlation Analysis. The relationship between the changes in protein structure profiles (in terms of amide I, amide II, ratio of amide I to amide II, α-helix, β-sheet or ratio of α-helix to β-sheet) and the changes in chemical and nutrient profiles (in terms of chemical composition, protein fractions, *in situ* rumen degradation kinetics, estimated protein intestinal digestibility and predicted protein supply to dairy cattle) in the canola meal and presscake samples were analysed using the PROC CORR procedure of SAS. The normality tests were performed using the UNIVARIATE procedure of SAS with options of NORMAL and PLOT.

Multiple Regression Analysis. In order to determine which protein molecular structure parameters (amide I, amide II, ratio of amide I to amide II, α-helix, β-sheet, ratio of α-helix to β-sheet) in the samples of canola meal and canola presscake are important in determining protein utilisation and availability to cattle, a multiple regression analysis with variable selection analysis was carried out using the PROC REG procedure of SAS with a model as follows:

Y = amide I + amide II + amide I to amide II ratio (R_I_II) + α-helix + β-sheet + α-helix to β-sheet ratio (R_a_β)

The model used a "STEPWISE" option with variable selection criteria: SLENTRY = '0.05', SLSTAY = '0.05'. All variables left in the final prediction models were significant at the $P < 0.05$ level. The residual analysis was carried out to test the regression model assumptions using the UNIVARIATE procedure of SAS with NORMAL and PLOT options.

Results and Discussion

Quantifying Protein Molecular Structure Profile in Canola Meal and Canola Presscake.

Heat-induced changes in characteristics of protein molecular structure. Goelema[27] had summarized that the heat treatment may results in protein denaturation (disorganization of the overall molecular shape of a protein), unfolding or uncoiling of a coiled or pleated structure, or the separation of the protein into its subunits, which may then unfold or uncoil. In our study, the processing conditions

did not change significantly the area of amide I between the two canola meal varieties or between canola meals and presscake (Table 1). However, the amide II area as well as the amide I to amide II ratio (R_I_II) was different ($P < 05$) between the brown-seed canola meal (CM_B) and the brown-seeded canola presscake (CPC_B). The R_I_II could be affected by the feed type, heat processing and even gene transformation.[28]

Even if tissues contain the same protein content, their nutritive value may be different if their α-helix to β-sheet ratios differ.[29] More specific, high percentage of β-sheet structure may partly cause low access to gastrointestinal digestive enzymes, which may result in a low protein value and low protein availability.[8] In this study a significant higher β-sheet value was observed for the yellow-seeded canola meal compared to the brown one. This result is in accordance, the lower rumen degradability and the higher intestinal digestibility found for the same yellow canola meal samples in a previous study.[15]

Processing conditions resulted in a significant higher value for α-helix and β-sheet for CPC_B in comparison with CM_B. Also, the R_a_β value was lower for CM_Y compared with the CPC_B. The lower values for CM_B could be partly explained due to processing conditions. Canola presscake was not exposed to the high temperature of the desolventizer-toaster, while during the process stage of desolventization-toasting the meal was heated to 95-115°C. [15]

The effects of heat processing on protein molecular structure characteristics are equivocal among studies and this might be due to different heating processing conditions. Yu et al.[3] found that roasting decreased the percentage of α-helix (from 47.1 to 36.1%), increased the percentage of β-sheet (from 37.2 to 49.8%), and decreased the α-helix-to-β-sheet ratio (1.3 to 0.7) of the golden flaxseed tissues. This is in contrast with the results obtained by Doiron et al.[29] where heating flaxseed at 120^0C for 40 and 60 min increased the α-helix to β-sheet ratio.

Multivariate Analysis of Heat-Induced Changes Protein Structural Spectra.

The multivariate spectral analyses (CLA and PCA) were applied when we study the molecular structure differences and functional groups. The cluster analysis is a multivariate analysis of which function performs an (agglomearative hierarchical) cluster analysis of an infrared spectra data set and displays the results of cluster analysis as dendrograms. In this study, the Ward's algorithm method was used without any prior paramerization of the spectral dat in the protein amides IR region. The success to apply multivariate CLA analysis has been reported previously[30] for the three feed inherent structures (structure 1, feed pericarp; structure 2, feed aleurone; structure 3, feed endosperm) and different varieties of canola; Doiran et al.[31] for flaxseed samples with heat treatment and Liu and Yu[32] for different genotypes of barley. On the other hand, the principal component analysis is a statistical data reduction method which transforms the original set of variables to a new set of uncorrelated variables called principal components. The first few principal components will typically account for high percentage variance (>95% variance). The purpose of PCA analysis is to derive a small number of independent linear combinations (principal components) of a set of variables that retain as much as of the information in the original variables as possible and displays the results as scatter plots between the components. Therefore, PCA can be used in order to allow the feed intrinsic structures to be distinguished and identifies features that differ between feed structures.

Figure 1a,1b,1c shows typical spectra, smoothed spectra and 2^{nd} derivative spectra in cotyledon layer Typical spectra (original spectra, smoothed spectra and 2^{nd} derivative spectra) of yellow canola meal, brown canola meal revealed by ATR-FT/IR molecular spectroscopy. It is hard to visually detect the spectral difference between the yellow and brown canola meal, while easier can be observed between canola meal and presscake.

The multivariate molecular spectral analyses (PCA and CLA) were applied to reveal the molecular structural difference between the yellow and brown canola meal varieties. Figure 2a shows the results of PCA and CLA analyses of molecular spectral (amide I and amide II region: *ca* 1700-1480 cm^{-1}) obtained from the samples of these two canola meal varieties. It was indicated that there were no significantly

molecular structural differences in the amide I and II region between the yellow and the brown canola meal, because they did not form two separate classes and also were not groped in separate ellipses. The scatter plot of the 1st principal component *vs* the 2nd principal component of PCA analysis of spectrum obtained from the yellow and brown canola meal samples is shown in Figure 4a. There the 1st and 2nd PCA explained % and % of the total variance, respectively.

The PCA analysis of spectrum obtained from the brown canola meal and presscake is shown in Figure 2b. The mixed dendrogram of brown canola meal and presscake showed a similarity of spectral data in their amide I and II regions, indicating that these two feedstuffs were not completely different in protein spectroscopic features. The PC1 and PC2 explained 93.59% and 4.92% of variation of spectral data, respectively.

Moreover, the molecular spectral comparison between canola meal (both yellow and brown) and canola presscake showed some overlap between them in the dendrogram (Figure 2c). However, there this was not the case in the scattered plot of PCA where showed two clearly distinguishable ellipses in which PC1 and PC2 explained 94.60% and 3.85% of variation of spectral data, respectively.

Correlations between Protein Structure Characteristics and Nutrient and Chemical Profiles.

Heat-Induced Changes in Amide I and Amide II Profiles in Relation to Nutrient and Chemical Profiles. Amide I and amide II bands is also known to have a great sensitivity to protein secondary structural changes.[19,20] Correlation between protein molecular structure and chemical and protein profiles, protein subfractions, total digestible nutrients, energy value, *in situ* degradation and intestinal protein digestibility canola feedstuffs used in this study is presented in Table 2. For chemical profiles, ether extract (EE) was found to be negatively correlated with the amide I and amide II (R = -0.97, $P < 0.05$) and dry matter tended to be negatively correlated to amide I to amide II ratio ((R = -0.94, $P = 0.05$). There was a significant positive correlation between R_I_II and soluble crude protein (SCP) (R = 1.00, $P < 0.05$). Moreover, a positive correlation was found between neutral acid-detergent insoluble crude protein (NDICP) and amide I (R = 0.95, $P = 0.051$) or amide II (R = 0.95, $P = 0.052$). However, no correlation (R = -0.97, $P > 0.10$) was observed between the amide I or amide II and the acid-detergent insoluble protein (ADICP) (Table 2).

For the Cornell net carbohydrate and protein system (CNCPS) protein subfractions, the results showed that the R_I_II had strong positive correlation with the PA (R = 0.99 $P < 0.01$) and with the slow degraded protein PB3 fraction (R = 0.99 $P < 0.01$). There were no correlations with the PB1, PB2, PC and true protein (the sum of PB1, PB2 and PB3).

For the *in situ* degradation parameters, the results indicated that the amide I and amide II had positive correlations with the degradable fraction and the degradation rate (R = 0.95 $P < 0.05$). No correlations were noted for the *in vitro* intestinal digestibility of rumen undegraded protein.

For the prediction of protein supply to dairy cattle, there were no significant correlations between any parameters of the DVE/OEB evaluation system and the protein structure in terms of amide I, amide II and their ratio (Table 4). On the other hand, the amide I as well as the amide II (R = 1.00 $P < 0.01$) were correlated with the $AMCP^{NRC}$ and the $AECP^{NRC}$ value was correlated with the amide I to amide II ratio (R = 0.98 $P < 0.05$). The metabolisable protein and the degraded protein balance based on NRC-2001 model were also significant correlated with the R_I_II.

Heat-Induced Changes in Protein Secondary Profiles in Relation to Nutrient and Chemical Profiles. There was a tended correlation between the neutral detergent fibre and the (R_a_β) (R = 0.80 $P = 0.057$) and a significant positive correlations with acid detergent fibre and lignin (Table 3). Others found a negative relationship between acid detergent insoluble crude protein and the ruminal and intestinal availability.[33,34] In this study, a positive correlation (R = 1.00 $P < 0.05$) between the α-helix and ADICP was found which indicates that higher α-helix value may result in lower ruminal and intestinal protein availability of canola meal or presscake.

Our results show that there was a positive correlation between protein structure α-helix to β-sheet ratio and *in situ* protein degradation in the rumen, which was in agreement with a published study by Samadi et

al.[35] on heat-treated whole canola seed. On the other hand, no correlation was found between the intestinal digestibility of rumen undegraded protein and α-helix to β-sheet ratio.

For the CNCPS protein subfractions the PB1 fraction and true protein tend to be correlated with the R_a_β (Table 3).

Furthermore, modelling results showed that although calculations equations were different between the DVE/OEB system and the NRC-2001 model, α-helix was positively correlated with both the $AMCP^{DVE}$ ($R = 0.98$ $P < 0.05$) and $AMCP^{NRC}$ ($R = 0.94$ $P < 0.05$). The α-helix to β-sheet ratio found to be negatively correlated with the $ARUP^{DVE}$ value and the DVE was tented to be correlated with the α-helix (Table 5). The prediction of protein supply based on NRC-2001 model showed that β-sheet was significantly positively correlated with the metabolisable protein and the degraded protein balance. The protein secondary structure characteristics had no correlation with the ruminally undegraded crude protein independently of the used evaluation system.

Prediction of Protein Digestive Characteristics from Protein Molecular Structure.

Multiple regression equations were successfully established based on the protein profiles and digestive characteristics and protein molecular structure parameters. The tested multiple regression model was: Y = amide I + amide II + amide I to amide II ratio (R_I_II) + α-helix + β-sheet + α-helix to β-sheet ratio (R_a_β). This analysis was carried out in order to explore the most suitable protein structure variable than could be used to predict nutrient supply to dairy cattle.

Using Protein Structure Parameters as a Predictor of Protein Profile, in Situ Rumen Degradation Kinetics, Estimated Intestinal Protein Digestibility and Predicted Nutrient Supply. For protein profiles the result showed that the ratio of α-helix to β-sheet was a better predictor for crude protein and neutral detergent insoluble protein, accounting for 79% and 68% of total variance, respectively (Table 7). For the CNCPS protein subfractions the ratio of amide I to amide II can be used as predictor of the slowly degradable protein fraction (PB3) with 74% of the variance being accounted for. These results were in accordance with the study of Liu et al.[28] on different cereals grains (wheat, corn and triticale) and their dried distillers grains with soluble.

For the *in situ* degradation kinetics, the ratio of α-helix to β-sheet was the only significant predictor for the degradation rate, degradable fraction, protein ruminal effective and rumen undegradable protein, while the same structure variable can be solely used to predict the estimated intestinal digestibility *in vitro* (Table 6).

In terms of protein supply based on the DVE/OEB system, the R_a_β sheet was the only variable left in the model for ENDP, $ARUP^{DVE}$ and DVE accounting for 71%, 81% and 81% of variation respectively (Table 7). Using the NRC-2001 model the ratio of amide I to amide II was a better predictor of the $AMCP^{NRC}$ and DPB, while the $ARUP^{NRC}$ and MP can be predicted by α-helix to β-sheet ratio.

Conclusions

In summary, the results of this study indicated that ATR-FT/IR molecular spectroscopy can be used in order to characterize feed structures at a molecular level rapidly and also that is it possible to relate feed functionality, digestive behaviour and nutrient utilization to the specific chemical make-up of intrinsic structures of the feed. This ATR-FT/IR molecular spectroscopy technology will provide us with a greater understanding of the plant-animal interface which is very important to economical and sustainable animal productions. However, a large scale *in vivo* research study is needed to test and verify the applicability of the protein molecular structural parameters investigated.

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Table 1. The structural characteristics of protein by using ATR-FT/IR molecular spectroscopy: comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*).

Item	Canola treatment			SEM	P value	Contrast
	CM-Yellow *B. Juncea* (n= 2 ×5 runs)	CM-Brown *B. Napus* (n= 2 ×5 runs)	CPC-Brown *B. Napus* (n= 2 ×5 runs)			CM *vs* CPC P value
Protein molecular structure spectra profiles (Unit: Absorbance)						
Amide I area	3.21[a]	2.89[a]	3.21[a]	0.157	0.262	0.414
Amide II area	1.13[ab]	0.93[b]	0.07[a]	0.074	0.046	0.090
Ratio amide I to amide II area	2.90[ab]	3.19[a]	2.70[b]	0.110	0.013	0.014
Protein secondary structure profile						
α-helix (height)	0.03[ab]	0.03[b]	0.04[a]	0.002	0.002	0.002
β-sheet (height)	0.04[a]	0.03[b]	0.04[a]	0.004	0.134	0.825
Ratio α-helix to β-sheet	0.86[b]	0.96[ab]	1.04[a]	0.033	0.002	0.003

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

Table 2. Correlation between protein structures (amide I, amide II and their ratio) and chemical protein and nutrient profiles of canola meal and canola presscake

	Protein molecular structure (amide I, amide II and their ratio)					
	Amide I		Amide II		Ratio of amide I to amide II	
Items	Correlation coefficient R	*P*	Correlation coefficient R	*P*	Correlation coefficient *R*	*P*
Chemical profiles (g kg^{-1} DM)						
DM[a]	-0.44	0.556	-0.45	0.555	-0.94	0.055
EE[b]	-0.97	0.034	-0.97	0.034	-0.16	0.836
NDF[c]	-0.21	0.692	-0.26	0.622	0.24	0.640
ADF[d]	-0.19	0.716	-0.14	0.791	0.03	0.951
ADL[e]	-0.23	0.666	-0.18	0.735	0.059	0.912
Protein profiles (g kg^{-1} DM)						
CP[f]	0.54	0.455	0.54	0.457	-0.74	0.263
NPN[g]	-0.50	0.503	-0.50	0.502	-0.92	0.081
SCP[h]	0.07	0.932	0.07	0.930	1.00	0.003
NDICP[i]	0.95	0.051	0.95	0.052	-0.18	0.820
ADICP[j]	0.91	0.272	0.91	0.265	0.88	0.311
Protein fractions (g kg^{-1} CP)						
PA[k]	0.02	0.984	0.02	0.983	0.99	0.008
PB1[l]	-0.76	0.244	-0.76	0.243	-0.70	0.301
PB2[m]	0.82	0.188	0.82	0.183	-0.45	0.545
PB3[n]	0.18	0.817	0.18	0.816	0.99	0.009
PC[o]	-0.70	0.297	-0.70	0.297	0.35	0.649
TP[p]	-0.07	0.890	-0.28	0.590	0.41	0.418
Digestible nutrients (NRC-2001 summary approach) (g kg^{-1} DM)						
tdCP[q]	0.13	0.799	-0.09	0.8626	0.33	0.5263
TDN_{1x}[r]	0.20	0.698	0.60	0.2086	-0.87	0.0239
In situ protein degradation kinetics (g kg^{-1} CP)						
K_d (h^{-1})[s]	0.95	0.048	0.95	0.049	-0.17	0.829
S[t]	-0.88	0.119	-0.88	0.118	-0.57	0.426
D[u]	0.95	0.049	0.95	0.048	0.38	0.625
EDCP[v]	-0.93	0.073	-0.93	0.073	-0.46	0.537
RUCP[w]	-0.76	0.238	-0.76	0.237	-0.68	0.320
Intestinal digestibility of RUCP (g kg^{-1}						

CP)						
IVCPD[x]	0.89	0.107	0.89	0.108	-0.32	0.679

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$). [a]DM, Dry Matter. [b]EE, Ether Extract (crude fat). [c]NDF, Neutral Detergent Fiber. [d]ADF, Acid Detergent Fiber. [e]ADL, Acid Detergent Lignin. [f]CP, Crude Protein. [g]NPN, Non-protein Nitrogen. [h]SCP, Soluble Crude Protein. [i]NDICP: Neutral Detergent Insoluble Crude Protein. [j]ADICP, Acid Detergent Insoluble Crude Protein. [k]TP, True protein = PB1 (g kg^{-1} CP) + PB2 (g kg^{-1} CP) + PB3 (g kg^{-1} CP). [l]PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN. [m]PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN. [n]PB2, intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC. [o]PB3, slowly degradable protein fraction, calculated as NDICP minus ADICP. [p]PC, fraction of undegradable protein, calculated as ADICP. [q]tdCP, digestible crude protein. [r]$TDN_{1X,}$ total digestible nutrients at maintenance estimated from NRC dairy model 2001. [s]$K_{d,}$ degradation rate. [t]S, potential soluble fraction in the *in situ* ruminal incubation. [u]D, potentially degradable fraction in the *in situ* ruminal incubation. [v]EDCP, Effectively degraded Crude Protein. [w]RUCP, Rumen Undegraded Crude Protein. [x]IVCPD, *in vitro* crude protein digestibility in small intestine.

Table 3. Correlation between protein structures (α-helix, β-sheet and their ratio) and chemical protein and nutrient profiles of canola meal and canola presscake

	Protein molecular structure (amide I, amide II and their ratio)					
	α-helix		β-sheet		Ratio of α-helix to β-sheet	
Items	Correlation coefficient *R*	*P*	Correlation coefficient R	*P*	Correlation coefficient *R*	*P*
Chemical profiles (g kg^{-1} DM)						
DM[a]	-0.21	0.791	-0.99	0.008	0.84	0.165
EE[b]	-0.93	0.071	-0.52	0.476	0.83	0.173
NDF[c]	0.01	0.988	-0.60	0.206	0.80	0.058
ADF[d]	0.11	0.830	-0.52	0.286	0.82	0.044
ADL[e]	0.07	0.900	-0.57	0.238	0.83	0.039
Protein profiles (g kg^{-1} DM)						
CP[f]	0.73	0.269	-0.42	0.578	-0.03	0.968
NPN[g]	-0.27	0.731	-0.99	0.008	0.88	0.125
SCP[h]	-0.18	0.819	0.89	0.110	-0.57	0.426
NDICP[i]	-0.35	0.772	-0.51	0.660	0.95	0.194
ADICP[j]	1.00	0.003	0.22	0.780	-0.65	0.354
Protein fractions (g kg^{-1} CP)						
PA[k]	-0.23	0.768	0.86	0.136	-0.53	0.472
PB1[l]	-0.58	0.416	-0.90	0.0964	0.94	0.058
PB2[m]	0.94	0.065	-0.07	0.931	-0.40	0.599
PB3[n]	-0.06	0.935	0.93	0.071	-0.65	0.347
PC[o]	-0.79	0.206	0.02	0.9767	0.40	0.603
TP[p]	-0.52	0.294	0.17	0.747	-0.80	0.054
Digestible nutrients (NRC-2001 summary approach) (g kg^{-1} DM)						
tdCP[q]	-0.32	0.533	0.38	0.461	-0.88	0.022
TDN_{1x}[r]	0.56	0.250	0.48	0.333	0.02	0.971
In situ protein degradation kinetics (g kg^{-1} CP)						
K_d (h^{-1})[s]	1.00	0.002	0.23	0.771	-0.65	0.346
S[t]	-0.74	0.259	-0.85	0.154	0.98	0.018
D[u]	0.86	0.139	0.70	0.299	-0.93	0.074
EDCP[v]	-0.81	0.185	-0.77	0.233	0.96	0.043

RUCP[w]	-0.59	0.406	-0.89	0.111	0.94	0.065
Intestinal digestibility of RUCP (g kg^{-1} CP)						
IVCPD[x]	0.98	0.023	0.08	0.924	-0.53	0.469

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

[a]DM, Dry Matter. [b]EE, Ether Extract (crude fat). [c]NDF, Neutral Detergent Fiber. [d]ADF, Acid Detergent Fiber. [e]ADL, Acid Detergent Lignin. [f]CP, Crude Protein. [g]NPN, Non-protein Nitrogen. [h]SCP, Soluble Crude Protein. [i]NDICP: Neutral Detergent Insoluble Crude Protein. [j]ADICP, Acid Detergent Insoluble Crude Protein. [k]TP, True protein = PB1 (g kg^{-1} CP) + PB2 (g kg^{-1} CP) + PB3 (g kg^{-1} CP). [l]PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN. [m]PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN. [n]PB2, intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC. [o]PB3, slowly degradable protein fraction, calculated as NDICP minus ADICP. [p]PC, fraction of undegradable protein, calculated as ADICP. [q]tdCP, digestible crude protein. [r]$TDN_{1X,}$ total digestible nutrients at maintenance estimated from NRC dairy model 2001. [s]$K_{d,}$ degradation rate. [t]S, potential soluble fraction in the *in situ* ruminal incubation. [u]D, potentially degradable fraction in the *in situ* ruminal incubation. [v]EDCP, Effectively degraded Crude Protein. [w]RUCP, Rumen Undegraded Crude Protein. [x]IVCPD, *in vitro* crude protein digestibility in small intestine.

Table 4. Correlation between protein structures (amide I, amide II and their ratio) and chemical protein and nutrient profiles of canola meal and canola presscake

	Protein molecular structure (amide I, amide II and their ratio)					
	Amide I		Amide II		Ratio of amide I to amide II	
Items	Correlation coefficient *R*	*P*	Correlation coefficient *R*	*P*	Correlation coefficient *R*	*P*
Modelling protein nutrients (DVE/OEB system) (g kg^{-1} DM)						
$AMCP^{DVE\ a}$	0.90	0.099	0.90	0.0995	-0.30	0.697
$ENDP^{b}$	-0.14	0.862	-0.14	0.863	0.48	0.516
$ARUP^{DVE\ c}$	0.20	0.709	0.05	0.919	0.13	0.811
DVE^{d}	0.83	0.170	0.83	0.171	-0.44	0.564
OEB^{e}	0.75	0.252	0.75	0.253	-0.55	0.447
Modelling protein nutrients (NRC-2001 model) (g kg^{-1} DM)						
$AMCP^{NRC\ f}$	1.00	<0.001	1.00	<0.001	0.161	0.839
$AECP^{g}$	-0.04	0.961	-0.04	0.962	0.98	0.025
$ARUP^{NRC\ h}$	-0.37	0.630	-0.37	0.632	0.84	0.161
MP^{i}	0.25	0.754	0.25	0.753	0.99	0.006
DPB^{j}	0.20	0.800	0.21	0.798	1.00	0.002

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

[a]$AMCP^{DVE}$, truly absorbed rumen synthesized microbial protein in the small intestine. [b]ENDP, endogenous protein losses in the digestive tract. [c]$ARUP^{DVE}$, truly absorbed bypass feed protein in the small intestine. [d]DVE, truly absorbed protein in the small intestine contributed by 1) feed protein escaping rumen degradation (RUP^{DVE}), 2) microbial protein synthesized in the rumen (MCP_{FOM}) and 3) a correction for endogenous protein losses in the digestive tract (ENDP). [e]OEB, reflects the difference between the potential microbial protein synthesis based on rumen degraded feed crude protein (CP) and that based on energy (rumen fermented OM) available for microbial fermentation in the rumen. [f]$AMCP^{NRC}$, truly absorbed rumen synthesized microbial protein in the small intestine. [g]AECP, truly absorbed endogenous protein in the small intestine. [h]$ARUP^{NRC}$, truly absorbed rumen undegraded feed protein in the small intestine. [i]MP, metabolizable protein (true protein that is digested post-ruminally and the component amino acid absorbed by the intestine) contributed by 1) ruminally undegraded feed CP, 2) ruminally synthesized microbial CP and 3) endogenous CP. [j]DPB^{NRC}, reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen

Table 5. Correlation between protein structures (amide I, amide II and their ratio) and chemical protein and nutrient profiles of canola meal and canola presscake

	Protein molecular structure (amide I, amide II and their ratio)					
	α-helix I		β-sheet		Ratio of α-helix to β-sheet	
Items	Correlation coefficient *R*	*P*	Correlation coefficient *R*	*P*	Correlation coefficient *R*	*P*
Modelling protein nutrients (DVE/OEB system) (g kg^{-1} DM)						
$AMCP^{DVE}$ [a]	0.98	0.020	0.09	0.906	-0.55	0.455
$ENDP^{b}$	-0.26	0.741	0.37	0.634	-0.19	0.809
$ARUP^{DVE}$ [c]	-0.55	0.451	-0.74	0.259	0.79	0.212
DVE^{d}	-0.20	0.709	0.51	0.304	-0.90	0.014
OEB^{e}	0.94	0.058	-0.051	0.951	-0.42	0.580
Modelling protein nutrients (NRC-2001 model) (g kg^{-1} DM)						
$AMCP^{NRC}$ [f]	0.96	0.037	0.54	0.465	-0.87	0.134
$AECP^{g}$	-0.28	0.716	0.83	0.172	-0.49	0.515
$ARUP^{NRC}$ [h]	-0.58	0.418	0.58	0.421	-0.16	0.839
MP^{i}	-0.00198	0.998	0.96	0.043	-0.71	0.290
DPB^{j}	-0.048	0.952	0.94	0.058	-0.68	0.322

SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

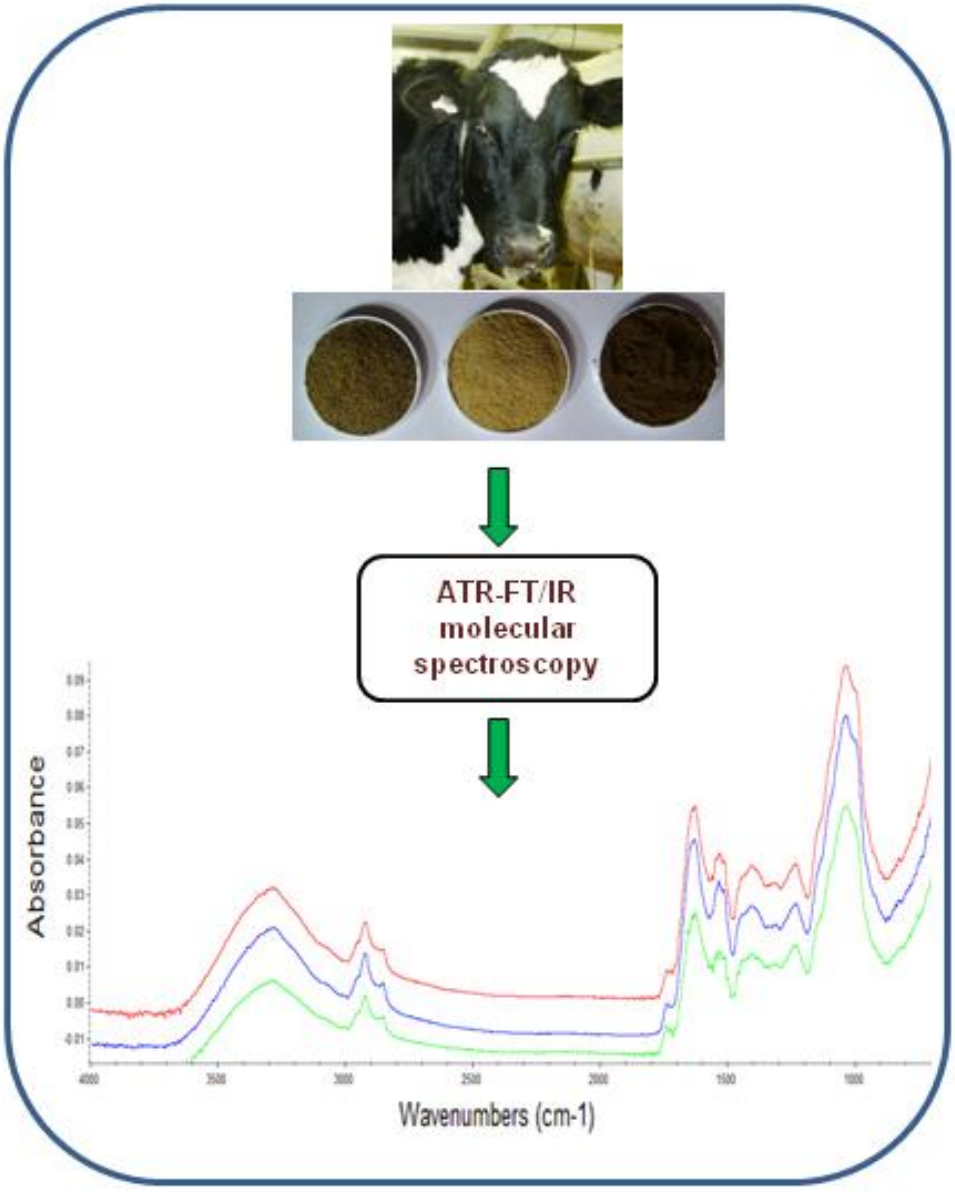
[a]$AMCP^{DVE}$, truly absorbed rumen synthesized microbial protein in the small intestine. [b]ENDP, endogenous protein losses in the digestive tract. [c]$ARUP^{DVE}$, truly absorbed bypass feed protein in the small intestine. [d]DVE, truly absorbed protein in the small intestine contributed by 1) feed protein escaping rumen degradation (RUP^{DVE}), 2) microbial protein synthesized in the rumen (MCP_{FOM}) and 3) a correction for endogenous protein losses in the digestive tract (ENDP). [e]DPB^{DVE}, reflects the difference between the potential microbial protein synthesis based on rumen degraded feed crude protein (CP) and that based on energy (rumen fermented OM) available for microbial fermentation in the rumen. [f]$AMCP^{NRC}$, truly absorbed rumen synthesized microbial protein in the small intestine. [g]AECP, truly absorbed endogenous protein in the small intestine. [h]$ARUP^{NRC}$, truly absorbed rumen undegraded feed protein in the small intestine. [i]MP, metabolizable protein (true protein that is digested post-ruminally and the component amino acid absorbed by the intestine) contributed by 1) ruminally undegraded feed CP, 2) ruminally synthesized microbial CP and 3) endogenous CP. [j]DPB^{NRC}, reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

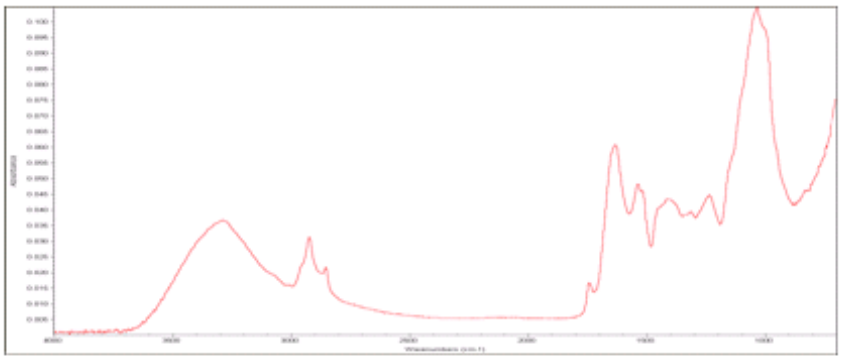
Table 6. Data obtained from multiple regression analysis used to find the most important variables to predict protein nutrient supply using protein molecular structural parameters in canola meal and canola presscake

Predicted variables (Y)	Variable(s) selection (variables left in the model with $P < 0.05$)	Prediction equations (test model: Y=a + b1 × x1 + b2 × x2 +...)	R^2	RSD	*P*
Chemical profiles	No variables met the 0.05 significant level for entry in the model				
Protein profiles (g kg^{-1} CP)					
CP (g kg^{-1} DM)[a]	Ratio of α-helix to β-sheet left in the model	CP= 781.78 - 403.67 × R_a_β	0.792	29.04	0.018
NDICP[b]	Ratio of α-helix to β-sheet left in the model	NDICP= - 152.82 + 309.90 × R_a_β	0.680	29.83	0.044
PB3[c]	Ratio of amide I to amide II left in the model	PB3= 3.40 + 30.10 × R_I_II	0.738	5.036	0.028
PB2[d]	No variables met the 0.05 significant level for entry in the model				
PB1[e]	No variables met the 0.05 significant level for entry in the model				
PA[f]	No variables met the 0.05 significant level for entry in the model				
PC[g]	No variables met the 0.05 significant level for entry in the model				
TP[h]	No variables met the 0.05 significant level for entry in the model				
Digestible nutrients (NRC-2001 summary approach) (g kg^{-1} DM)					
tdCP[i]	Ratio of α-helix to β-sheet left in the model	tdCP= 876.08 - 524.53 × R_a_β	0.767	81.10	0.022
TDN_{1X}[j]	Ratio of amide I to amide II left in the model	TDN_{1X}= 1335.37 - 213.90 × R_I_II	0.759	67.77	0.024
In situ protein degradation kinetics (g kg^{-1} CP)					
K_d (h^{-1})[k]	Ratio of α-helix to β-sheet left in the model	K_d= -6.54 + 14.10 × R_a_β	0.769	1.085	0.022
D[l]	Ratio of α-helix to β-sheet left in the model	D= 1365.82 - 511.18 × R_a_β	0.787	37.26	0.018
EDCP[m]	Ratio of α-helix to β-sheet left in the model	EDCP= 154.29 + 362.058 × R_a_β	0.785	26.59	0.019
RUP[n]	Ratio of α-helix to β-sheet left in the model	RUP= 845.71 - 362.05 × R_a_β	0.785	26.59	0.019
S[o]	No variables met the 0.05 significant level for entry in the model				

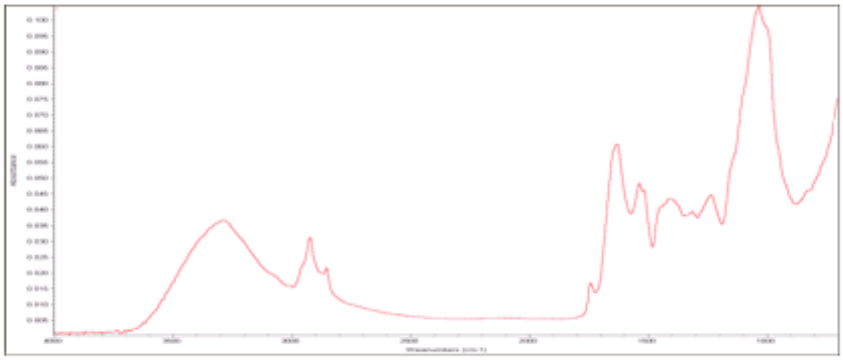
Intestinal digestibility of RUP (g kg^{-1} CP)					
IVCPD[p]	Ratio of α-helix to β-sheet left in the model	IVCPD= 1645.30 - 957.56 × R_a_β	0.759	75.68	0.024

[a]CP, Crude Protein. [b]NDICP, Neutral Detergent Insoluble Crude Protein. [c]PB3, slowly degradable protein fraction, calculated as NDICP minus ADICP. [d]PB2, intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC. [e]PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN. [f]PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN. [g]PC, fraction of undegradable protein, calculated as ADICP. [i]tdCP, digestible crude protein. [j]TDN_{1X}, total digestible nutrients at maintenance estimated from NRC dairy model 2001. [k]K_d, degradation rate. [l]D, potentially degradable fraction in the *in situ* ruminal incubation. [m]EDCP, Effectively degraded Crude Protein. [n]RUCP, Rumen Undegraded Crude Protein. [o]S, potential soluble fraction in the *in situ* ruminal incubation. [p]IVCPD, *in vitro* crude protein digestibility in small intestine.

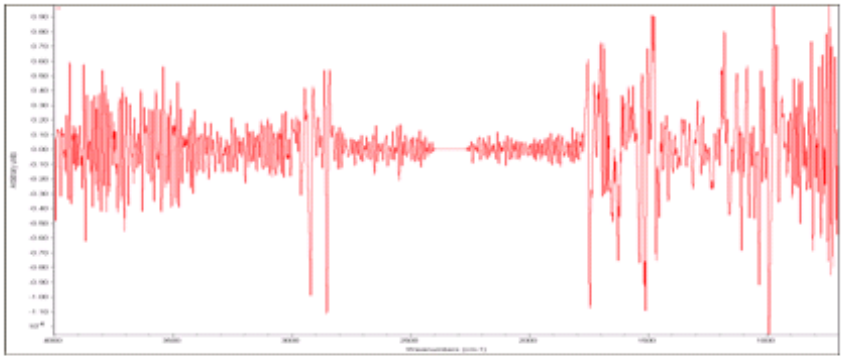

ATR-FT/IR
molecular
spectroscopy
Absorbance
Wavenumbers (cm-1)



A: Yellow canola meal (*B. Juncea*) (Original spectra)

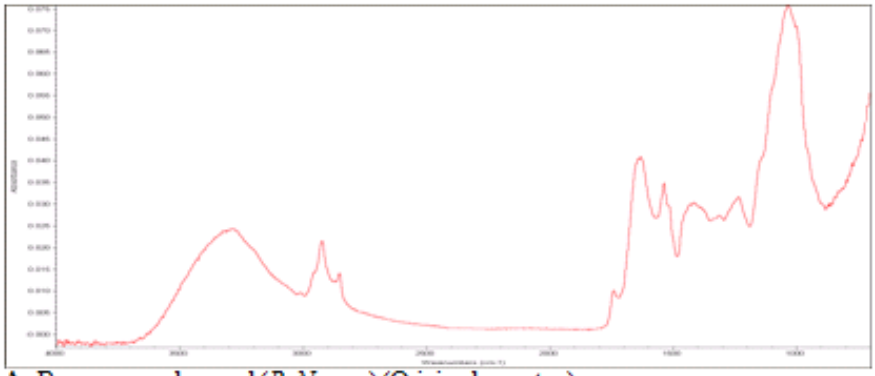


B: Yellow canola meal (*B. Juncea*) (Smoothed spectra)

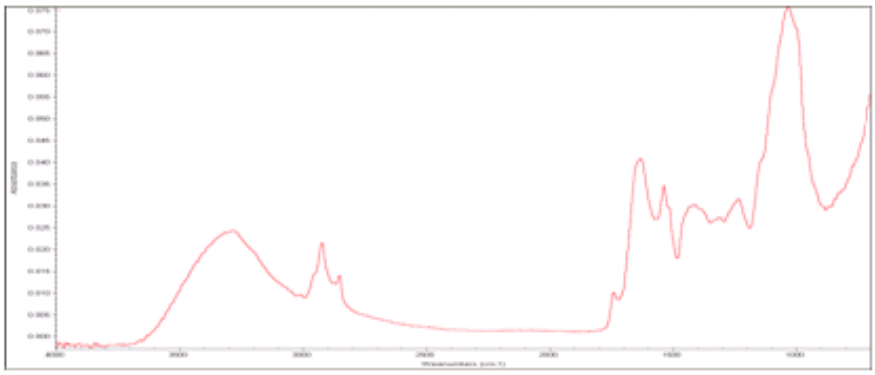


C: Yellow canola meal (*B. Juncea*) (2nd derivative spectra)

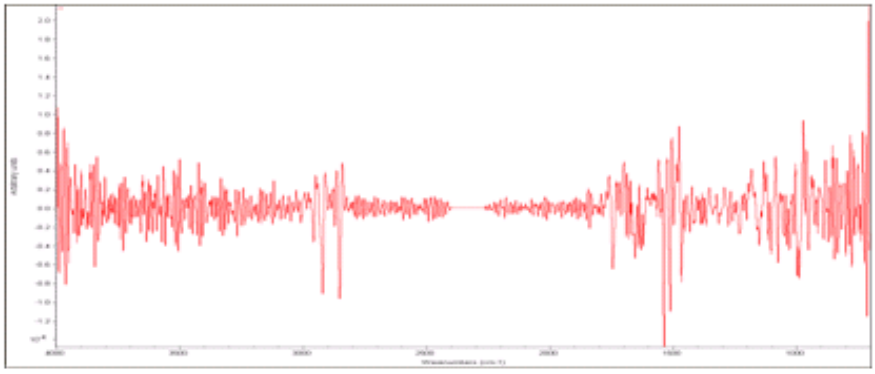
Figure 1a. Typical spectra (original spectra, smoothed spectra and 2nd derivative spectra) of yellow canola meal (1a: CM_Y: *B. Juncea*), brown canola meal (1b: CM_B: *B. Napus*), and canola brown presscake (1c: CPC_B: *B. Napus*).



A: Brown canola meal (*B. Napus*) (Original spectra)

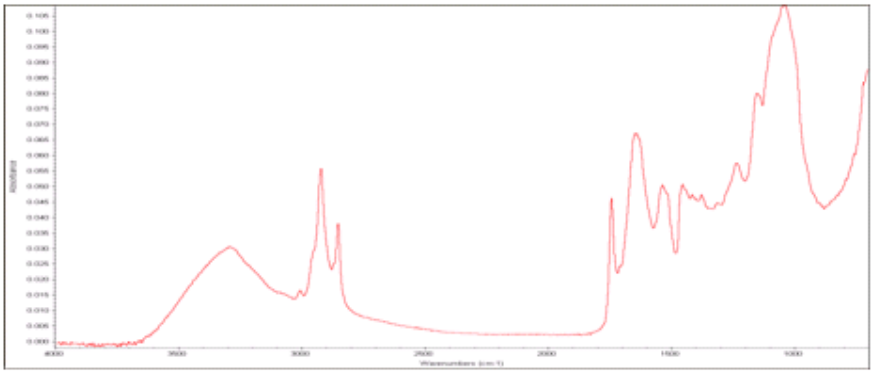


B: Brown canola meal (*B. Napus*) (Smoothed spectra)

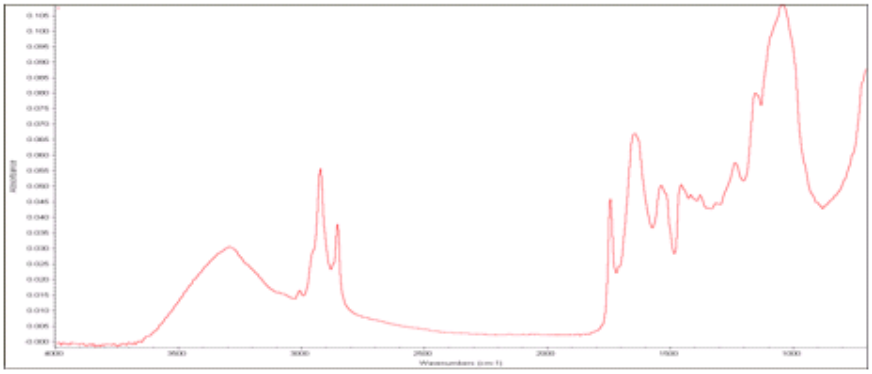


C: Brown canola meal (*B. Napus*) (2nd derivative spectra)

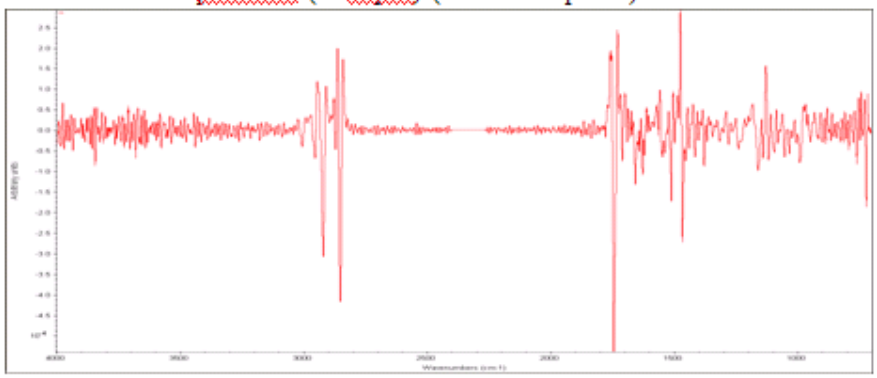
Figure 1b. Typical spectra (original spectra, smoothed spectra and 2nd derivative spectra) of yellow canola meal (1a: CM_Y: *B. Juncea*), brown canola meal (1b: CM_B: *B. Napus*), and canola brown presscake (1c: CPC_B: *B. Napus*).



A: Brown canola presscake (*B. Napus*) (Original spectra)

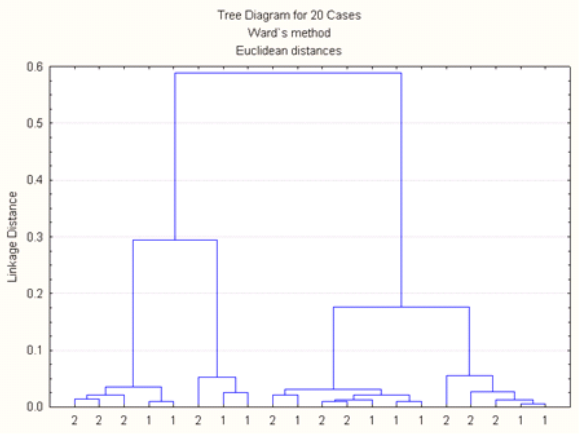


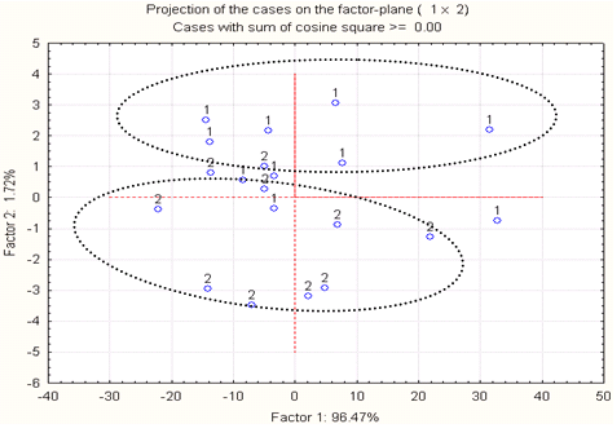
B: Brown canola presscake (*B. Napus*) (Smoothed spectra)



C: Brown canola presscake (*B. Napus*) (2nd derivative spectra)

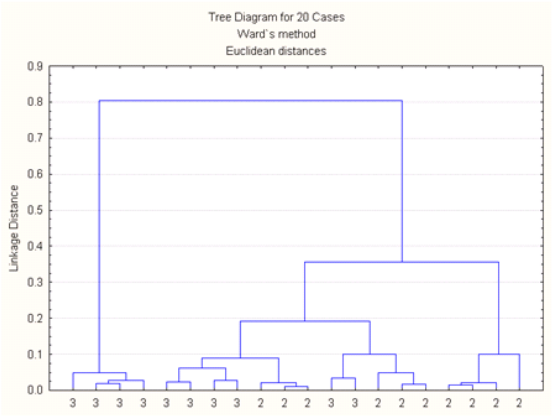
Figure 1c. Typical spectra (original spectra, smoothed spectra and 2nd derivative spectra) of yellow canola meal (1a: CM_Y: *B. Juncea*), brown canola meal (1b: CM_B: *B. Napus*), and canola brown presscake (1c: CPC_B: *B. Napus*).

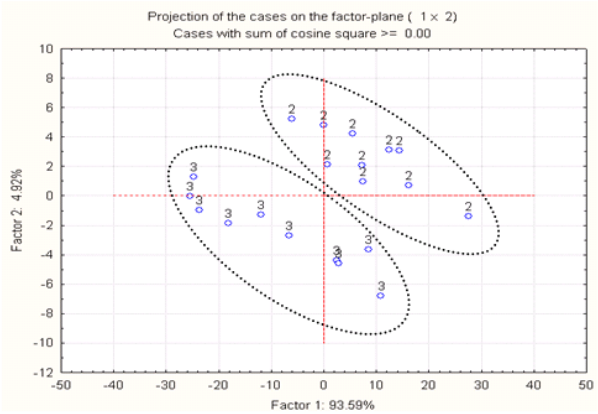




I: 1 = CM_Y (*B. Juncea*) *vs* 2 = CM_B (*B. Napus*). Cluster (CLA) spectral analysis: (1) select spectral region: amide I and amide II regions (*ca* 1700-1480 cm^{-1}); (2) distance method: Euclidean; (3) cluster cluster method: Ward's algorithm.	I: 1 = CM_Y (*B. Juncea*) *vs* 2 = CM_B (*B. Napus*). Scatter plot of the 1st principal component *vs* the2nd principal component. The 1st and 2nd principal component explains 96.47% and 1.72% of the total variance, respectively

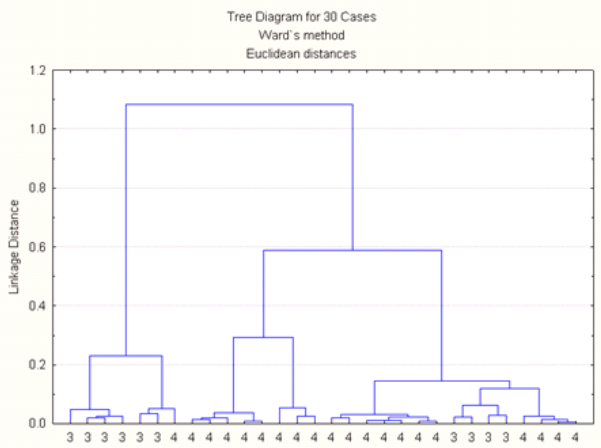
2a).



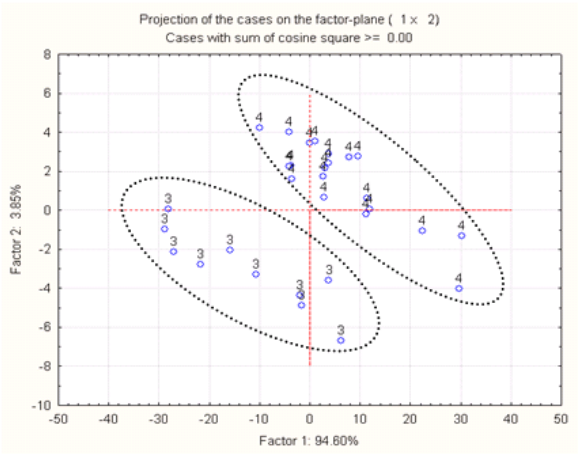


II: 2 = CM_B (*B. Napus*) *vs* 3 = CPC_B (*B. Napus*). Cluster (CLA) spectral analysis: (1) select spectral region: amide I and amide II regions (*ca* 1700-1480 cm^{-1}); (2) distance method: Euclidean; (3) cluster cluster method: Ward's algorithm.	II: 2 = CM_B (*B. Napus*) *vs* 3 = CPC_B (*B. Napus*). Scatter plot of the 1st principal component *vs* the2nd principal component. The 1st and 2nd principal component explains 93.59% and 4.92% of the total variance, respectively

2b).



III: 3 = CPC_B (*B. Napus*) *vs* 4 = CM average (CM_Y, CM_B).
Cluster (CLA) spectral analysis: (1) select spectral region: amide I and amide II regions (*ca* 1700-1480 cm^{-1}); (2) distance method: Euclidean; (3) cluster cluster method: Ward's algorithm.



III: 3 = CPC_B (*B. Napus*) *vs* 4 = CM average (CM_Y, CM_B)
Scatter plot of the 1st principal component *vs* the2nd principal component. The 1st and 2nd principal component explains 94.60% and 3.85% of the total variance, respectively

Figure 2. Multivariate molecular spectral analyses of amide I and amide II regions of the feedstuffs used in this study: (I) comparison of yellow canola meal (CM_Y: B. Juncea) and brown canola meal (CM_B: B. Napus); (II) comparison of brown canola meal (CM_B: B. Napus), and canola presscake brown (CPC_B: B. Napus); (III) comparison of canola meal (CM_Y and CM_B) and canola presscake (CPC_B).

Program II:

Research Program in Newly Developed Yellow-Seeded and Black-Seeded Canola Lines

The Program I includes the following three main projects.

Project 4: MAGNITUDE DIFFERENCES IN BIOACTIVE COMPOUNDS, CHEMICAL FUNCTIONAL GROUPS, FATTY ACID PROFILES, NUTRIENT DEGRADATION AND DIGESTION, MOLECULAR STRUCTURE AND METABOLIC CHARACTERISTICS OF PROTEIN IN NEWLY DEVELOPED YELLOW-SEEDED AND BLACK-SEEDED CANOLA LINES (COMPLETED) *

A version of this project has been published: K. Thedoridou, X. Zhang, S. Vail, P. Yu. 2015. Magnitude Differences in Bioactive Compounds, Chemical Functional Groups, Fatty Acid Profiles, Nutrient Degradation and Digestion, Molecular Structure, and Metabolic Characteristics of Protein in Newly Developed Yellow-Seeded and Black-Seeded Canola Lines. J. Agric. Food Chem (USA). 2015, 63: 5476–5484 (DOI: 10.1021/acs.jafc.5b01577) (*as Supervisor, PI and Corresponding Author).

Project Details

Abstract:

Recently new lines of yellow-seeded (CS-Y) and black-seeded canola (CS-B) have been developed with chemical and structural alteration through modern breeding technology. However, no systematical study was found on the bioactive compounds, chemical functional groups, fatty acid profiles, inherent structure, nutrient degradation and absorption, metabolic characteristics between the newly developed yellow-seeded and black-seeded canola lines. This study aimed to systematically characterize chemical, structure, and nutrition features in these canola lines. The parameters accessed include: bioactive compounds and anti-nutrition factors, chemical functional groups, detailed chemical and nutrient profiles, energy value, nutrient fractions, protein structure, degradation kinetics, intestinal digestion, truly intestinal protein supply, and feed milk value. The results showed that the CS-Y line was lower ($P \leq 0.05$) in neutral detergent fibre (122 vs. 154 g/kg DM), acid detergent fiber (61 vs. 99 g/kg DM), and lignin (58 vs. 77 g/kg DM), non-protein nitrogen (56 vs 68 g/kg DM), and acid detergent insoluble protein (11 vs. 35 g/kg DM) than the CS-B line. There was no difference in fatty acid profiles except C20:1 Eicosenoic acid content (omega-9) which was in lower in the CS-Y line ($P < 0.05$) compared to the CS-B line. The glucosinolate compounds differed ($P<0.05$) in terms of 4-pentenyl, phenylethyl, 3-CH3-indolyl, and 3-butenyl glucosinolates (2.9 vs. 1.0 µmole/g) between the CS-Y and CS-B lines. For bioactive compounds, total polyphenole tended to be different (6.3 vs. 7.2 g/kg DM) but there were no differences in erucic acid and condense tannins with averages of 0.3 and 3.1 g/kg DM, respectively. When portioning protein into five subfractions, the significant differences were found in PA, PB1 (65 vs. 79 g/kg CP), PB2, and PC fractions (10 vs. 33 g/kg CP), indicated protein degradation and supply to small intestine differed

between two new lines. In terms of protein structure spectral profile, there were no significant differences in functional groups of amides I and II, alpha-helix and beta-sheet structure as well as their ratio between two new lines, indicating no difference in protein structure make-up and conformation between the two lines. In terms of energy values, there were significant differences in total digestible nutrient (TDN: 149 vs. 133 g/kg DM), metabolizable energy (ME: 58 vs. 52 MJ/kg DM) and net energy for lactation (NEL: 42 vs. 37 MJ/kg DM) between CS-Y and CS-B lines. For in situ rumen degradation kinetics, the two lines differed in soluble fraction (S: 284 vs. 341 g/kg CP), potential degradation fraction (D: 672 vs. 590 g/kg CP), effective degraded organic matter (EDOM: 710 vs. 684 g/kg OM) but no difference in degradation rate. The yellow line (CS-Y) had higher digestibility of rumen bypass protein in the intestine than the black line (566 vs. 446 g/kg of RUP, $P<0.05$). Modeling nutrient supply results showed that microbial protein synthesis (MCP: 148 vs. 171 g/kg DM) and rumen protein degraded balance (DPB: 108 vs. 127 g/kg DM) were lower in the CS-Y line but there were no differences in total truly digested protein in small intestine (DVE) and feed milk value (FMV) between the two lines. In conclusion, the new yellow lines had different nutrition, chemical and structure features compared to the black lines. The yellow line of CS-Y provided a better nutrient utilization and availability.

Keywords: bioactive compounds, nutrition and anti-nutrition profile, protein structure, fatty acid, energy value, biodegradation and digestion

Introduction

Oilseeds and their products are the most valuable agricultural crops in world trade. Nowadays, canola is considered the second most abundant source of edible oil in the world[1] whilst canola meal is widely used as a protein source in livestock feeding.[2] Canadian breeders in 1970's developed canola, containing low "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and of "glucosinolates" in the meal portion (<30 μmol of any one or any combination of the four aliphatic glucosinolates in its defatted meal).[3]

The high erucic acid, glucosinolates, and condensed tannins in rapeseed could have adverse effects on animal health. Specifically, erucic acid, a fatty acid with the configuration C22:1 n-9, has been known to cause heart lesions in experimental animals.[4] If animal eat high amounts of this acid, it can result in "fat deposits infiltrating their heart walls".[5] On the other hand, glucosinolates have negative impact on palatability, feed efficiency, iodine uptake, and weight gains in animals[6]. As to condensed tannins, the high content of condensed tannin affects biological functions such as intake, digestion.

At the same time, the improvement of the quality of canola seed and its by-products (i.e. canola meal) has been attempted, by increasing the digestibility of the hull and/or reducing the hull proportion in the meal.

Canola varieties consist of the yellow and the black (dark brown)-seeded varieties. Hulls from yellow-seeded have lower fiber but higher protein and oil than the black-seeded.[7,8]

Apart from the chemical composition of oilseed crops, their protein secondary structure profiles may also influence the quality and absorption of proten.[9-12] Therefore, to understand crop's digestive behavior, nutritional value, it is important to look at the protein structure eg. 2nd strucutre.[10]

However, studies aiming at evaluating the nutritional value, for ruminants, of *B. napus* yellow seed varieties, especially their degradation kinetics and protein molecular structure are scarce. In our study, we hypothesized that these newly developed yellow and black canola seed varieties, might be different in terms of their nutritional value and nutrient degradation characteristics as well as their protein's inherent molecular structural makeup.

Objective

This study aimed to systematically characterize chemical, structure, and nutrition features in these yellow- and black-seeded canola lines. The parameters accessed include: bioactive compounds and anti-nutrition factors, functional groups, detailed chemical and nutrient profiles including fatty acid

profiles, energy value, nutrient fractions, protein structure, degradation kinetics, intestinal digestion, protein supply, and predicted feed milk value.

Material and Methods

New Lines of Yellow-Seeded and Black-Seeded Canola

Yellow and black canola seeds (*B. napus*) were supplied by the *Agriculture and Agri-Food Canada,* Saskatoon Research Center, Canada and used as feed sources. The yellow seeded line was called YN01-429 and the black seeded line was a breeding line named N07-1374 which has a contrasting pedigree to YN01-429. Seed were harvested from plots grown in 2010 and 2011 in adjacent trials about 1500 m apart at the Agriculture and Agri-Food Canada Saskatoon Research Centre in Saskatoon, Canada.

For the black seeded line, a plot size of 1.9 m^2 had approximately 100 seeds planted mid-may each season. At maturity, seeds was directly combined from the plot. A plot size of 7.4 m^2 was used to grow the yellow seeded line from approximately 800 seeds planted in mid-May and plots were combined about two weeks after swathing. Seeding dates were four and one day apart for the two different trials in 2010 and 2011, respectively. For all samples, seed from two different replications in each experiment of the same line were combined to form the samples used in this study. Adjusted yields ranged from 2200 to 3950 kg/ha for N07-1374 and 2400 to 2700 kg/ha for YN01-429 in 2010 and 2011, respectively. Agronomically, the lines were very similar with flowering times and maturity ratings ranging from one to three days and two to six days, respectively with N07-1374 having a slightly later maturity rating.

Rumen in Situ Incubation and degradation kinetics

The Animal Care Committee at Uni of Sask approved this trial.[13] The detailed 'gradual addition/all out' procedure was reported previously.[14]
In situ degradation kinetics of different nutrient (DM, CP, OM, NDF) were determined. [15-18]

Digestibility of Rumen Bypass Protein in small intestine.

The digestibility of rumen bypass crude protein was determined according to the 3-step in vitro procedure as described by Calsamiglia and Stern.[19]

Chemical Profiles

The residues of the nylon bags, from both two experimental runs, were collected and pooled according to the incubation time, treatment and run. Samples were analysed for chemical and nutritional profiles using the methods from AOAC [20] and Van Soest et al [21]

To prevent inaccurately high NDF value, the samples was incubated in acetone for two hours to extract fat. The methods for determining the contents of soluble crude protein (SCP) and non-nitrogen protein (NPN) were previously described by Theodoridou and Yu.[22]

Oil and Fatty Acids

Oil content was analysed using AOAC (method 920.39) and for fatty acid profile (method 969.33 for preparation and 996.06 for qualification). Briefly, accurately weighed ground seeds samples (0.40g) were put into a labelled Mojonnier flask and then 110 mg pyrogallic acid, 2.0 mL C21:0 (internal standard), 2.0 mL ethanol and 10.0 mL HCl (8.3M) were added. For complete extraction, the solution was mixed well very 10 min on vortex mixer and then cooled to room temperature (25°C). As for the methylation procedure, chloroform (3mL) and diethyl ether (3mL) were used to dissolve the extracted fat and then evaporated again in the water bath (40°C) under N stream in a glass vial. After dryness completed, cooled the vial to room temperature (25°C) and added H_2O (5.0mL), hexane (1.0mL) and Na_2SO_4 (1g) to allow layer separation. The top layer was transferred to another vial containing 1g Na_2SO_4 for gas chromatography analysis (Agilent6890, Agilent Technologies Inc., CA, USA). The column used in this study was a DB 225 capillary column (0.10mm ID, 10min length and 0.10um film thickness).The column

temperature program was setup as follows: Initial temperature: 35°C, 25°C/min to 195°C, 3°C/min to 205°C, 8°C/min to 230°C. The detector temperature was at 250°C. The analysis was repeated if the error was higher than 5% between two replications.

Glucosinolates, Polyphenols, and Condensed Tannins

Glucosinolate profile in the samples were analysed using the CGA method.[25] The method of of Total polyphenols and condensed tannins (CT: HCl-butanol procedure) in samples were determined according to Slinkard and Singleton [26] and Reed[27]. All samples were analysed in duplicate; the analysis was repeated if the error was higher than 5%.

Energy Values and Protein Sub-Fractions

Energy values were estimated using a summative approach as suggested by the NRC 2001 dairy. The Cornell System of CNCPS was applied to partition protein fractions.[28]

Molecular Structure Profile Explored Using Molecular Spectroscopy

Molecular spectroscopic experiments were performed at Molecular Spectroscopy Lab in Feed Science Division at APS of Uni of Sask. Attenuated total reflectance-Fourier-transformed infrared-vibration spectroscopy (JASCO FT/IR-ATR-4200, Jasco Inc., Easton, MD Corp., Tokyo, Japan) were carried out. Samples were placed in the refrigerator to minimize sticking while grinding. Later on they were fed slowly into the grinder to further prevent sticking during the grinding process and were finely ground using a fit with a 0.5 mm screen. Five subsamples from each sample were analyzed with thirty-two scans per sample in the 4.000 to 800 cm^{-1}. The functional spectral bands and their intensity of ratio calculation (eg. amide I, II, 2nd structures) were according to the previously published study.[29]

Using the Dutch System to Predict Potential Protein Nutrient Supply and Feed Milk Value (FMV)

Potential protein nutrient supply was predicted based on the Dutch system [30-33] The FMV was determined based on the metabolizable protein from the NRC 2001 model.

Statistical Analysis

The data were statistically analysed using the Mixed Model procedure of SAS (version 9.3) (SAS Institute 1999). For chemical and nutrient data, the model was: $Y_{ij} = \mu + V_i + e_{ij}$, The model used for rumen and intestinal data was: $Y_{ijk} = \mu + V_i + R_k + e_{ijk}$, where, Y_{ijk} is the dependent variable *ijk*, μ is the population mean, V*i* was the fixed effect of canola seed type, R_k was a random effect of the in situ run, and e_{ijk} was the random error. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

Results and Discussion

Chemical Characterization

The results on the chemical profiles of the yellow (CS-Y), and black-type (CS-B) canola seeds are presented in Table 1. No significant differences were found between the CS-Y and the CS-B for the basic nutrients. For carbohydrate profiles, CS-Y was lower in ADF (60.7 vs 98.6 g/kg DM), ADL (8.1 vs 43.5 g/kg DM) whereas tend to be lower in NDF (122.1 vs 154.5 g/kg DM) ($P \leq 0.10$) (Table 1). It has been reported that dietary fiber content in yellow-seeded samples was significantly lower than the black-seeded types and this is due to "the bigger seed size, the lower contribution of the hull fraction to the total seed mass, and the lower fiber content of the hull fraction for the yellow seeded canola".[7] Moreover, Abraham and Bhatia[34] mentioned that yellow-seeded Brassica genotypes "have a thinner and translucent seed coat, resulting in a lower hull proportion with a bigger embryo and consequently greater oil and protein contents in the seeds". Moreover, hull consist of low molecular weight carbohydrates cellulose, pectin, lignin and other compounds.[35] So in this study the significantly notable lower ADL in the CS-Y compared

to the CS-B could be higher digestible hull in the yellow seed.

Regarding the protein profile of the feedstuffs used in this study, CS-Y had lower ($P < 0.05$) NPN (g/kg DM or g/kg SCP) and ADICP (g/kg DM). A trend toward significance observed when ADICP expressed as g/kg CP) and in that case the value was lower for the yellow canola compared to the black one (50.6 vs 149.4 g/kg CP). There was no difference in the crude protein as well as the sugar content between the two types of canola seed. The fact that the seed coat color did not results in different CP content is in accordance with previous study targeting brown and yellow carinata seed.[36]

Bioactive Compounds, Anti-Nutrition Factors, and Fatty Acids Profiles

Data on the fatty acid profile and the bioactive compounds present in yellow and black canola seeds are summarized in Table 2. Canola oil is recognised for having a relatively low level of saturated fatty acids (7% or less) and significant amounts of essential fatty acids.[37] In this study, there was no difference in the erucic acid level . Among the fatty acids the C20:1 Eicosenoic acid content (omega-9) was lower ($P < 0.05$) for the CS-Y than for the CS-B.

There are many studies focusing on the phenolics compounds in crops such as soybean, sunflower and sorghum, however, knowledge of these compounds in canola remains fragmentary even though canola contains about five times as much as phenolics compounds as soybeans.[38] The predominant phenolics groups present in canola seed are phenolics acids and condensed tannins. The last one, presents in from150 to 300 g/kg DM, with higher level in black-seeded varieties.[39-40] In this study, there was no significant differences in the CT content while the total polyphenols tended to be lower for the yellow than the black seed (6.3 vs 7.2 g/kg DM). According to Shahidi et al.,[41] phenolics compounds (i.e. CT) contribute to dark colour of canola seed and their by-products.

Glucosinolates are natural antinutritional compounds found in most brassica seeds and plants such as mustard, brussels sprouts, cabbage and califlower. The presence of relatively high levels of certain glucosinolates in traditional types of rapeseed hindered the use of rapeseed meal as a protein supplement in animal nutrition. The composition of glucosinolates in canola seeds depends on the seed variety.[42,3] In our study was found that specific glucosinolates compounds present in a higher amounts at the yellow than at the black canola seeds. The 3-butenyl, the phenylethyl and the 3-CH3-indolyl exist in a significantly higher amount in the CS-Y compared to the CS-B.

Fractionation of Protein

Protein and sub-fractions are shown in Table 3. Canola yellow type was significantly lower in the PA (3.9 vs 5.8 g/kg CP) and the unavailable PC fraction [24] (10.1 vs 33.2 g/kg CP) fractions compared to the black one. Since fraction PC in the feed represents the unavailable part of protein which cannot be used effectively by the ruminants, therefore, our results implied that the yellow canola seeds were expected to show comparatively better protein utilization than the brown seeds.

Energy Values in Newly Developed Lines

Truly digestible nutrients[18] and energy content of yellow and black canolavariety are summarized in Table 4. The CS-Y was higher ($P < 0.05$), compared to CS-B, in total digestible non-fiber carbohydrate (367.1 vs 277.3 g/kg DM) and total digestible nutrients (149.4 vs 133.5 g/kg DM). The total digestible NDF tended to be higher for the CS-Y compared to the CS-B (21.1 vs 7.8 g/kg DM).

On the other hand, the energy values (DE_{1X}, DE_{3X}, $ME_{3X,}$ NE_{L3X}) were higher ($P < 0.05$) for the yellow compared to the black-seeded canola. This result might be due the tendency for lower fiber content and reduced hull of CS-Y compared to the CS-B.

In Situ Rumen Incubation

The effect of canola seed variety on rumen fractions, rate of degradation and effective degradability of nutrients is presented in Table 5. Comparing CS-Y with CS-B, there was no differences in the rumen degradability, soluble fraction and degradation rate of dry matter. However, the *D* fraction of dry matter in canola yellow-seeded (727.6 g/ kg DM) was higher than the black one (650.7 g/kg DM). The degradable

fraction of organic matter (Table 5) was higher for the CS-Y compared to the CS-B (799.0 vs 642.8 g/kg OM).

The results on the in situ rumen protein degradation study showed that the EDCP (g/kg CP) of CS-Y was similar to that of CS-B. However, the potential soluble fraction was lower ($P < 0.05$) and the degradable fraction was higher ($P < 0.05$) for the yellow compared to the black-seeded canola.

The NDF degradability depends on the proportion of its main components (i.e. cellulose, hemicellulose and lignin). Lignin, as well as render cellulose and hemicellulose that are associated with it, is known to be highly indigestible.[20] In our study, the lower ($P < 0.05$) ADF and ADL content of yellow-seeded and the tendency of the NDF content to be lower, compared to the black one, was expected to be in accordance with a higher EDNDF (g/kg NDF) for CS-Y. However, there was no difference between CS-Y and CS-B concerning the degradations characteristics of neutral detergent fiber.

Intestinal Digestibility of Rumen Undegradated Protein

In vitro crude protein intestinal digestibility of CS-Y was higher ($P < 0.05$) compared to that of CS-B (565.9 vs 445.6 g/kg CP of rumen undegradable protein) (Table 6). The lower undegradable protein fraction (PC) and the higher intestinal protein digestibility of yellow canola compared to the black-seeded one, implies that yellow type is a better source of intestinal digestible proteins for ruminants.

The absorbance of crude protein in the ruminant's small intestine has been reported to be negatively correlated ($r = -0.81$) with the acid detergent insoluble N content in canola meal[43]. This is in agreement with the results obtained in our study, where the higher protein intestinal digestibility of CS-Y, compared with the CS-B, was in accordance to its lower ($P < 0.05$) ADICP value.

Protein Metabolic Characteristics and Feed Milk Value

The protein metabolic characteristics and the feed milk value are shown in Table 7. As described before, the microbial protein production is estimated on the FOM basis in the Dutch system. Therefore, similarity in FOM between the yellow and the black resulted in similar values in MCP_{FOM} and AMCP.

In comparison with the black-seeded canola, the microbial protein that synthesized in the rumen and based on the rumen degraded feed protein was significantly lower for the CS-Y in accordance with its trend ($P < 0.10$) for lower OEB value.

In our study, the fact that the OEB value[30] tended to be lower ($P < 0.10$) for the yellow-seeded canola, indicates this seed type may results in a better nitrogen availability and utilisation by the animals.

The feed milk value for both two types of canola seed was not different ($P > 0.05$), which is expected to have similar efficiency for milk production.

Protein Molecular Structures of Yellow and Black-Seeded Canola

The profiles of protein molecular structure, of yellow and black-seeded canola as defined with attenuated total reflectance-Fourier-transformed infrared-vibration spectroscopy (ATR-FT/IR), are presented in Table 8. The research show that protein 2nd structure affect th protein by changing protein quality and biofunctions.[29,44-49] Depending on the α-helix to β-sheet ratios, tissues of the same protein content may differ in their nutritional value.

Figure 1 shows typical spectra, smoothed spectra and 2nd derivative spectra of CS-Y and CS-B revealed by ATR-FT/IR molecular spectroscopy. In the present study, no significant differences were observed for the protein's inherent molecular structural make up, between the CS-Y and the CS-B.

In contrast with our results, Theodoridou et al.,[50] concluded that the amide I, II areas, the β-sheet and the ratio of amide I to amide II were higher ($P < 0.05$) for black compared to the yellow-seeded canola. One possible reason for this variation between the two studies, might be the fact that another analytical technique, called synchrotron-based vibrational FTIR microspectroscopy (SR-FTIRM) [51, 52], has been employed in their study.

Conclusion

In summary, the results of this study indicated that the yellow-seeded canola was lower in ADF, ADL, NPN and ADICP content compared to the black one. The yellow-seeded canola had higher values in the total digestible nutrients content, net energy, digestible and metabolizable energy. There were no differences for the protein's inherent structural spectral characteristics. The findings of the present study show that the breeding of yellow type of canola is a promising route to reducing fiber and hull content, while at the same time increasing the level of oil. The feed protein availability and utilization by the animal might be higher when yellow type canola is fed to the animals, since the in vitro intestinal digestibility was found to be higher for the yellow-seeded canola compared to the black one. On the other hand, the yellow-seeded canola tended to have a lower degraded balance, compared to the black one, which suggests a better nutrient utilization and less output of potentially polluting nitrogen. This is something that needs to be consider since environmental friendly rations are nowadays very important in animal nutrition.

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Table 1. Chemical profiles: Comparison between yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
Basic chemical				
Dry matter	944.7	939.6	1.07	0.078
Ash (g/kg DM)	44.8	41.0	6.82	0.735
Carbohydrate profile (g/kg DM)				
Carbohydrate	314.2	323.8	11.19	0.607
Neutral Detergent Fiber	122.1	154.5	5.40	0.051
Acid Detergent Fiber	60.7	98.6	2.96	0.012
Acid Detergent Lignin	8.1	43.5	1.84	0.005
Nitrogen free Neutral Detergent Fiber	58.2	77.2	7.56	0.218
Nitrogen free Acid Detergent Fiber	50.1	63.3	5.98	0.259
Sugars (g/kg DM)	60.1	59.9	8.63	0.987
Protein Profile				
Crude Protein (g/kg DM)	207.9	237.5	7.22	0.102
Soluble Crude Protein (g/kg DM)	69.5	84.9	3.13	0.074
Soluble Crude Protein (g/kg CP)	333.9	357.6	5.57	0.095
Non-protein Nitrogen (g/kg DM)	55.6	68.0	1.88	0.043
Non-protein Nitrogen (g/kg CP)	267.0	218.8	58.59	0.620
Non-protein Nitrogen (g/kg SCP)	55.5	68.0	1.87	0.042
Neutral Detergent Insoluble Crude Protein (g/kg DM)	64.0	77.3	10.08	0.449
Acid Detergent Insoluble Crude Protein (g/kg DM)	10.6	35.4	3.81	0.044
Neutral Detergent Insoluble Crude Protein (g/kg CP)	309.6	326.9	56.29	0.848
Acid Detergent Insoluble Crude Protein (g/kg CP)	50.6	149.4	18.90	0.066

Table 2. Oil content, fatty acids, glucosinolates, and bioactive compounds in yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
Chemical compounds				
Erucic acid (g/kg DM)	0.2	0.4	0.07	0.184
Either Extract (EE, g/kg DM)	473.3	441.3	1.98	0.199
Fatty acid composition (mg FA/g)				
C12 Lauric	0.00	0.2	0.11	0.423
C14 Myristic	0.55	0.7	0.05	0.293
C16 Palmitic	33.7	39.0	1.58	0.141
C16:1n7 Palmitoleic	3.2	3.3	0.21	0.764
C17 Margaric	1.2	1.5	0.10	0.168
C18 Stearic	16.6	16.0	0.81	0.602
C18:1n9 Oleic	534.7	527.1	18.25	0.797
C18:1 Octadecenoic	25.3	25.80	3.33	0.918
C18:2 Linoleic	187.0	184.9	6.38	0.834
C18:3n3 alpha-Linolenic	74.9	84.0	3.56	0.211
C20 Arachidic	5.6	5.8	0.11	0.333
C20:1 Eicosenoic	11.3	12.0	0.11	0.049
C20:2n6 Eicosadienoic	0.7	0.65	0.08	0.699
C22 Behenic	3.0	3.3	0.15	0.293
C22:1n9 Erucic	0.2	0.40	0.07	0.184
C24 Lignoceric	1.2	1.2	0.04	0.423
C24:1n9 Nervonic	1.0	1.50	0.16	0.155
Others	2.4	3.9	0.46	0.148
Total Fatty Acids	902.0	910.7	5.17	0.356
Total Saturates	61.7	67.4	1.88	0.165
Total monounsaturates	575.6	570.0	14.45	0.811
Total Polyunsaturates	262.5	269.5	9.73	0.659
Total Omega 3	74.9	84.0	3.56	0.211
Total Omega 6	187.6	185.5	6.45	0.839
Total Omega 9	547.2	541.0	17.96	0.830
Glucosinolates (umoles/g)				
3-butenyl	2.9	1.0	0.08	0.004
4-pentenyl	0.4	0.7	0.05	0.058
2-OH-3-butenyl	2.6	2.4	0.37	0.860
2-OH-4-pentenyl	0.7	0.1	0.05	0.609
CH3-thiobutenyl	0.1	0.8	0.01	0.292
Phenylethyl	0.3	0.8	0.02	0.021
CH3-thiopentenyl	0.1	0.1	0.02	0.184
3-CH3-indolyl	0.3	0.2	0.01	0.011
4-OH-3-CH3-indolyl	3.4	3.2	0.12	0.508
Bioactive compounds (g/kg DM)				
Total Polyphenols	6.3	7.2	1.61	0.056
Condensed Tannins	3.0	3.2	0.22	0.592

Table 3. Protein and carbohydrate fractions portioned by Cornell Net Carbohydrate and Protein system (CNCPS) in yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
Protein fractions (g/kg CP)				
PA[a]	3.9	5.8	0.26	0.036
PB1[b]	65.6	79.2	2.87	0.080
PB2[c]	870.1	842.6	6.37	0.092
PB3[d]	50.4	39.4	11.04	0.555
PC[e]	10.1	33.2	3.60	0.045
True Protein[f] (g/kg CP)	986.1	961.1	3.79	0.043
Protein fractions (g/kg true protein)				
PB1	66.2	79.9	2.90	0.080
PB2	878.5	850.6	6.43	0.092
PB3	50.8	39.8	11.14	0.555
Protein fractions (g/kg DM)				
PA	0.8	1.3	0.08	0.042
PB1	13.0	17.7	0.108	0.090
PB2	171.0	188.0	7.12	0.233
PB3	9.8	8.8	1.92	0.736
PC	2.0	7.4	0.69	0.031

[a]PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN. [b]PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN. [c]PB2, intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC. [d]PB3, slowly degradable protein fraction, calculated as NDICP minus ADICP. [e]PC, fraction of undegradable protein, calculated as ADICP. It contained the proteins associated with lignin and tannins and/or heat-damaged proteins such as Maillard reaction products. [f]True protein (TP), PB1 (% CP) + PB2 (% CP) + PB3 (% CP).

Table 4. Truly digestible nutrients and estimated energy value: Comparison between yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
Truly digestible fractions (g/kg DM; NRC 2001)				
digestible non-fiber carbohydrate (tdNFC)	367.1	277.3	11.43	0.031
digestible crude protein (tdCP)	192.4	209.8	6.87	0.215
digestible fatty acid (tdFA)	437.1	404.6	11.72	0.189
digestible neutral detergent fiber (tdNDF)	21.1	7.8	2.54	0.066
Total digestible nutrients (TDN_{1x})[a]	149.4	133.5	1.94	0.029
Predicted energy value at production intake level (3x) for dairy cattle (MJ/kg DM; NRC 2001)				
Digestible energy (DE_{3x})[b]	59.9	54.0	0.81	0.037
Metabolizable energy (ME_{3x})[c]	57.9	51.8	0.85	0.037
Net energy for lactation (NE_{L3x}) [d]	42.0	37.3	0.71	0.042

[a]$TDN_{1X,}$ total digestible nutrients at maintenance estimated from NRC dairy model 2001. [b]$DE_{3X,}$ digestible energy three times maintenance estimated from the NRC dairy model 2001. [c]$ME_{3X,}$ metabolizable energy at three times maintenance estimated from the NRC dairy model 2001. [d]$NEL_{3X,}$ net energy for lactation at three times maintenance estimated from the NRC dairy model 2001. [e]$DE_{1X,}$ digestible energy at maintenance.

Table 5. In situ rumen degradation characteristics of dry matter, crude protein, organic matter and neutral detergent fiber. Comparison between yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
In situ rumen dry matter degradation characteristics				
S[a] (g/kg DM)	272.4	297.1	10.65	0.152
D[b] (g/kg DM)	727.6	650.7	13.24	0.006
T0[c] (/h)	0.9	0.9	0.16	0.958
K_d[d] (/h)	9.0	9.3	0.56	0.743
Rumen undegraded dry matter (RUDM, g/kg DM)	291.3	310.9	8.33	0.149
Effectively degraded dry matter (EDDM, g/kg DM)	708.7	689.2	8.33	0.149
In situ rumen organic matter degradation characteristics				
S (g/kg OM)	281.1	301.7	11.24	0.242
D (g/kg OM)	799.0	642.8	1.39	0.008
T0 (/h)	1.0	0.8	0.19	0.589
K_d (/h)	8.91	9.0	0.504	0.914
Rumen undegraded organic matter (RUOM,g/kg OM)	289.5	315.3	8.36	0.073
Effectively degraded organic matter (EDOM, g/kg OM)	710.4	684.8	8.36	0.073
In situ rumen crude protein degradation characteristics				
S (g/kg CP)	285.9	341.1	12.59	0.021
D (g/kg CP)	672.8	590.2	13.20	0.005
T0 (/h)	0.4	0.5	0.19	0.765
K_d (/h)	12.2	12.8	1.09	0.684
Rumen undegraded crude protein (RUCP, g/kg CP)	257.6	249.9	8.87	0.565
Effectively degraded crude protein (EDCP, g/kg CP)	742.5	750.1	8.87	0.565
In situ rumen neutral detergent fiber degradation characteristics				
S (g/kg NDF)	125.2	255.8	7.442	0.283
D (g/kg NDF)	874.8	689.4	7.00	0.134
T0 (/h)	1.1	1.0	0.21	0.718
K_d (/h)	9.0	8.7	0.64	0.732
Rumen undegraded neutral detergent fiber (RUNDF, g/kg NDF)	349.4	336.4	29.47	0.771
Effectively degraded neutral detergent fiber (EDNDF, g/kg NDF)	650.6	663.6	29.47	0.771

[a]S, potential soluble fraction in the in situ ruminal incubation. [b]D, potentially degradable fraction in the in situ ruminal incubation. [c]T0, lag time. [d]K_d, degradation rate.

Table 6. In vitro crude protein digestibility in the small intestine (IVCPD), on the 12h residue determined using three fistulated dairy cattle: Comparison between yellow and black-type of canola seed (*B. Napus*)

Item	CS-Yellow	CS-Black	SEM	*P* value
Protein digestibility in the small intestine (g/kg CP in ruminally incubated residues)				
IVCPD[a]	565.9	445.6	27.86	0.022

[a]IVCPD, in vitro crude protein digestibility in small intestine.

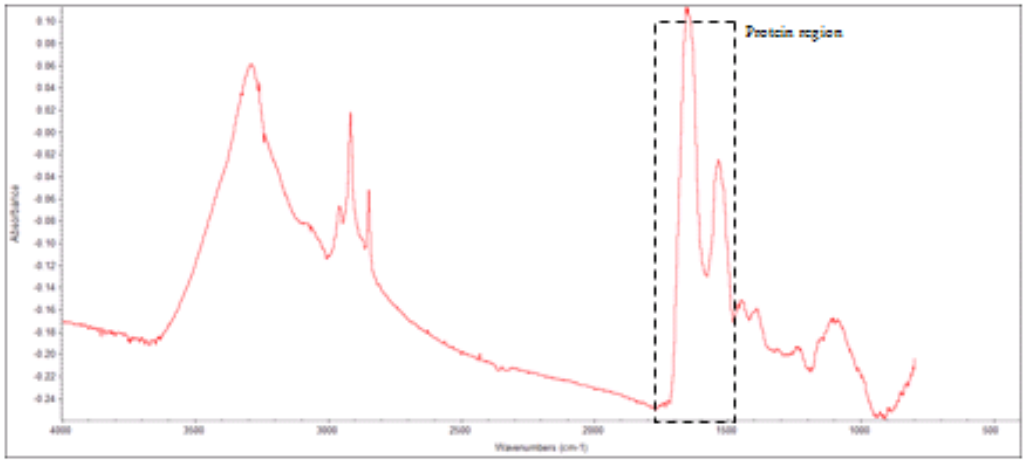
Table 7. Predicted values of potential nutrient supply to dairy cattle from yellow and black-type of canola seed (*B. Napus*) using the Dutch DVE/OEB system

Item (g/kg DM)	CS-Yellow	CS-Black	SEM	*P* value
Absorbable microbial protein synthesis in the rumen ($AMCP^{DVE}$)				
FOM^{a}	272.2	297.1	8.46	0.174
$MCP_{FOM}{}^{b}$	40.8	44.6	1.27	0.174
$MCP_{RDP}{}^{DVEc}$	148.45	171.47	3.398	0.041
$AMCP^{DVEd}$	26.04	28.41	0.809	0.174
Endogenous protein in the small intestine (ENDP)[e]				
ENDP	12.64	12.87	0.595	0.810
Truly absorbable rumen un-degraded protein in small intestine ($ARUP^{DVE}$)				
RUP^{DVEf}	59.45	65.98	4.149	0.382
$ARUP^{DVEg}$	33.64	29.74	4.787	0.622
Total truly digested protein in small intestine (DVE value)				
DVE^{h}	47.05	45.29	3.741	0.770
Degraded protein balance (OEB value)				
DPB^{DVEi} (MCP_{FOM} - MCP_{RDP})	107.61	126.92	4.504	0.094
Feed Milk Value[j]				
FMV (kg milk per kg feed)	0.96	0.92	0.076	0.769

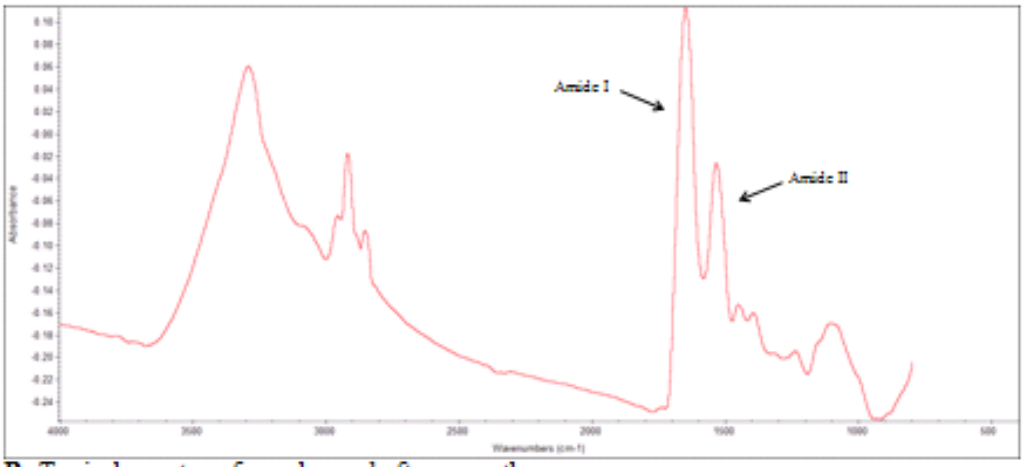
[a]FOM, organic matter fermented in the rumen. [b]$MCP_{FOM,}$ microbial protein synthesized in the rumen based on available energy. [c]$MCP_{RDP}{}^{DVE}$, microbial protein synthesized in the rumen based on rumen degraded feed crude protein. [d]$AMCP^{DVE}$,truly absorbed rumen synthesized microbial protein in the small intestine. [e]ENDP, endogenous protein losses in the digestive tract. [f]RUP^{DVE}, ruminally undegraded feed CP, calculated according the formula in DVE/OEB system. [g]$ARUP^{DVE}$, truly absorbed bypass feed protein in the small intestine. [h]DVE, truly absorbed protein in the small intestine contributed by 1) feed protein escaping rumen degradation (RUP^{DVE}), 2) microbial protein synthesized in the rumen (MCP_{FOM}) and 3) a correction for endogenous protein losses in the digestive tract (ENDP), DVE = (AMCP + ARUP – ENDP). [i]DPB^{DVE}, reflects the difference between the potential microbial protein synthesis based on rumen degraded feed crude protein (CP) and that based on energy (rumen fermented OM) available for microbial fermentation in the rumen. [j]FMV, reflects the efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (source NRC, 2001) and protein composition in milk is assumed 33 g protein per 1000 g milk.

Table 8. Protein molecular structure spectral profiles : Comparison between yellow (*B. Napus*) (CS-Y) and black (*B. Napus*) (CS-B) canola seeds

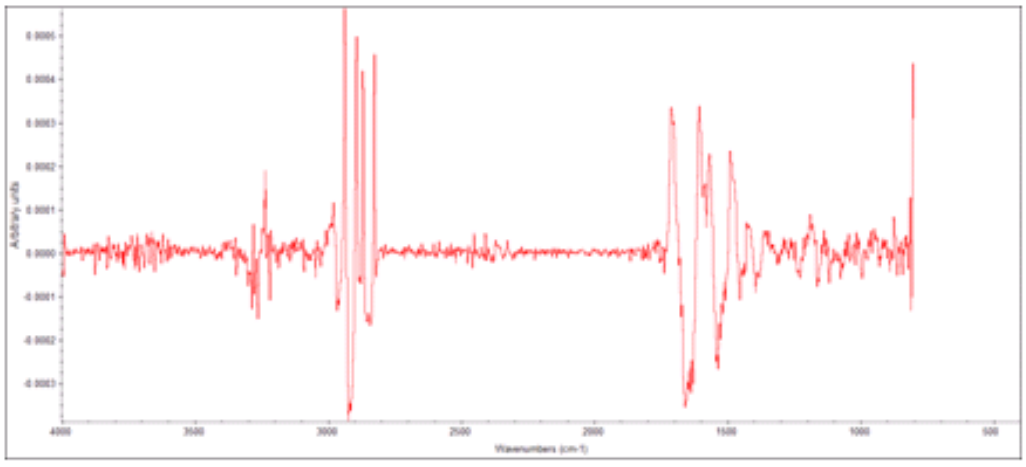
Endosperm region				
Item (Unit: IRAbsorbance)	CS-Yellow	CS-Black	SEM	*P* value
Protein molecular structure spectra profiles				
Amide I area	4.63	4.87	0.155	0.308
Amide II area	1.87	2.00	0.066	0.204
Ratio amide I to amide II area	2.48	2.44	0.043	0.501
Amide I height	0.06	0.07	0.002	0.246
Amide II height	0.04	0.04	0.001	0.409
Ratio amide I to amide II height	1.75	1.79	0.027	0.320
Protein secondary structure profile				
a-helix (height)	0.06	0.06	0.002	0.421
β-sheet (height)	0.06	0.06	0.002	0.538
Ratio a-helix to β-sheet	1.05	1.06	0.011	0.686



A: Typical spectra of canola seed



B: Typical spectra of canola seed after smooth



C: Typical 2nd derivative spectra of canola seed after smooth

Figure 1. Typical biomolecular spectrum of canola seed in the region ca. 4.000 to 800 cm^{-1}, showing the chemical functional group of protein amide I and II.

TOC: Typical spectra of Yellow and brown cannola seeds (ca. 1718-1488 cm^{-1})

Project 5: PROTEIN AND ENERGY METABOLIC CHARACTERISTICS AND NUTRIENT SUPPLY, TO RUMINANTS, FROM NEWLY DEVELOPED CANOLA LINES, AS PREDICTED USING THE NRC-2001 MODEL AND THE PDI SYSTEM (COMPLETED) *

A version of this project has been written as a shortcommunication: Katerina Theodoridou and Peiqiang Yu. 2016. Protein and energy metabolic characteristics and nutrient supply, to ruminants, from newly developed canola lines, as predicted using the NRC-2001 model and the PDI system. Manuscript Draft is Ready (*as supervisor, PI, corresponding author).

Project Details

Abstract:

Nowadays, the goal in agriculture sector, is the adoption of best practice in feeding management to improve the protein use efficiency and reduce the ecological footprint of the production of animal products As ruminant feed may influence the quality of animal products (i.e. meat and milk), and animal's productivity, there is a need to systematically evaluate the nutritive value of each feed. Especially about feed protein, the level of microbial protein synthesis and the amount of digestible protein in the intestine are important determinants of the response and efficiency with which dietary nitrogen is used for milk production. These points are taken into account in the most advanced protein evaluation systems, such as the NRC-2001 model, the DVE/OEB system and the PDI system. The objectives of this study were to (1) identify differences in the metabolic characteristics of protein and energy between newly developed canola lines, (yellow and black), modeled for dairy cattle, (2) determine the extent of ruminal and intestinal digestion and absorption of the protein, (3) determine feed milk value, and (4) compare the two evaluation systems in modeling nutrient supply, the National Research Council (NRC) 2001 model, and the protein truly digestible in the small intestine system (PDI). The results demonstrated that the PDI system predicted lower values for all the nutrient supply parameters studied compared to the NRC-2001 model. Independent of the feed evaluation system used in this study, both of them indicated that the microbial protein synthesis in the rumen based on the available protein and the rumen degraded protein absorbed in the small intestine, were lower for the yellow compared to the black-seeded canola.

Keywords: Canola seed, Metabolic characteristics, Nutrient supply, Ruminant

Introduction

Nowadays, due to a rapid growing world population, global demand for animal products is increasing. As a consequence, the agriculture sector faces multiple challenges: in one hand has to produce more food, with high nutritional quality in order to feed a growing population, and on the other hand has to adopt more efficient and sustainable livestock production methods. So the goal is the adoption of best practice in feeding management to improve the protein use efficiency and reduce the ecological footprint of the production of animal products (Herrero et al. 2009; Cao and Li, 2011). As ruminant feed may influence the quality of animal products (i.e. meat and milk), and animal's productivity, there is a need to systematically evaluate the nutritive value of each feed. The response and efficiency with which dietary protein is used for milk production, is determined mainly by the level of microbial protein synthesis and the amount of digestible protein in the intestine. These points are taken into account in the most advanced protein evaluation systems, such as the NRC-2001 model, the DVE/OEB and the PDI system. To our knowledge, no study exists in the literature to determine the protein metabolic characteristics, potential

nutrient supply and feed milk value of canola varieties, by employing and comparing the two evaluation systems, the NRC-2001 model and the PDI system. Although the basic framework of these evaluation systems (NRC-2001, PDI) is similar, some of the units and factors used to calculate the nutrient values differ.

The hypothesis of this study was that there is a variety effect on the protein and energy metabolic characteristics, protein digestibility and feed milk value, between the newly developed yellow and black canola lines. Also it is hypothesized that the two evaluation systems will yield different estimates when used to predict the parameters above.

Objective

The objectives were to (1) identify differences in the metabolic characteristics of protein and energy between the yellow and black-seeded canola, modeled for dairy cattle, (2) determine the extent of ruminal and intestinal digestion and absorption of the protein, (3) determine feed milk value, and (4) compare the two evaluation systems in modeling nutrient supply, namely, the National Research Council (NRC) 2001 model, and the protein truly digestible in the small intestine system (PDI).

Material and Methods

Seed samples

Yellow and black canola seeds (B. napus) were supplied by the Agriculture and Agri-Food Canada, Saskatoon Research Center, Canada and used as feed sources. Yellow and black canola seeds were collected from two different harvest years 2010 and 2011.

Rumen in situ incubation

Rumen incubations were performed according to the 'gradual addition/all out' schedule, as described previously by Yu et al. 2000 and Theodoridou and Yu (2012).

Rumen degradation kinetics

In situ rumen degradation kinetics of dry matter (DM), crude protein (CP), organic matter (OM) and neutral detergent fibre (NDF) were determined using the first-order kinetics equation described by Ørskov and McDonald (1979) and modified by Robinson et al. (1986) and Dhanoa (1988).This procedure was described previously Theodoridou and Yu (2012, 2013).

Nutrient Supply with the PDI system

The principle of the PDI system was provided by Verity and Geay (1987) and INRA (1978) and the quantifying calculations were described lately by Theodoridou and Yu (2013a). A short explanation will be presented in order to follow the principles of this system. The PDI content of an animal feed is the sum of two fractions: 1) PDIA: the dietary protein undegraded in the rumen, but truly digestible in the small intestine and 2) PDIM: the microbial true protein which is truly digestible in the small intestine. Each feed is characterised by two PDIM values. 1) PDIMN, which corresponds to the amount of microbial protein that could be synthesized in the rumen from the degraded dietary N, when energy and others nutrients are not limiting 2) PDIME, which corresponds to the amount of microbial protein that could be synthesized from the energy available in the rumen, when degraded N and other nutrients are not limiting. So the feed value can be calculate as PDIN = PDIA + PDIMN and PDIE = PDIA + PDIME. The PDI values are obtained from four individual feed characteristics: 1) CP content, 2) degradability of crude protein (RDP^{PDI}) obtained from the rumen incubation procedure, 3) fermentable organic matter content (FOM) calculated from the total digestible organic matter (DOM) content after subtraction of the contents of ether extract and undegradable dietary protein in the feed and fermentation products in silage, 4) true intestinal digestibility (TId) of rumen undegraded dietary true protein (RUP^{PDI}).

Nutrient Supply with the NRC-2001 model

The detailed concepts and formulas of the NRC-2001 model have been described previously (Theodoridou and Yu, 2013a) and so the following is a brief explanation in order to understand its basic principles and how to predict protein supply to the small intestine of dairy cows as a result of feeding canola seed. Based on this model, the metabolizable protein (MP) is composed of three important protein sources. The MP is contributed by: (1) ruminally undegraded feed CP (RUP^{NRC}), (2) ruminally synthesized microbial CP (MCP), and (3) rumen endogenous CP (ECP) and calculated as MP (g/kg of DM) = $ARUP^{NRC}$ + $AMCP^{NRC}$ + AECP, where AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine.

The degraded protein balance (DBP^{NRC}), reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on energy (discounted TDN) available for microbial fermentation in the rumen.

Feed Milk Value

Based on metabolic characteristics of protein from the NRC-2001 system and PDI model, the feed milk values were determined. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 and protein composition in milk is assumed 33 g protein per 1000 g milk.

Statistical analysis

Statistical analyses were performed using the Mixed Model procedure of SAS (version 9.3) (SAS Institute, 1999). The model used for the analysis was: $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable $_{ij}$; μ was the population mean for the variable; T_i was a fixed effect of seed type (i = 2; black canola seed and yellow canola seed), each harvest year being used as replications and e_{ij} was the random error associated with the observation $_{ij}$. The normality test to check the analysis model assumptions was performed using Proc Univariate with Plot and Normal options. Regression analysis among the models was performed using the REG procedure of SAS. The significance of differences between means was assessed using Tukey's test. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

Results and Discussion

Protein Supply to Dairy Cows using the PDI system

Prediction of the potential nutrient supply to dairy cow from canola seed using the PDI system is shown in Table 1. In relation to its significantly lower microbial protein that could be synthesized in the rumen from the degraded dietary nitrogen (PDIMN), the value of microbial protein synthesis in the rumen was significantly lower for the CS_Y than that for the CS_B. The highest ($P < 0.05$) value of the digestibility in the small intestine of the undegraded dietary protein was obtained for CS_Y compared to CS_B. Also, the balance between microbial protein synthesis, from available rumen degradable protein and potential energy in the rumen (DPB), was lower for CS_Y compared to CS_B ($P < 0.05$). This is in agreement with another study, where they predicted the nutrient supply by using the DVE/OEB evaluation system, and found that the yellow had a lower DPB than the brown B. carinata seed (Xin at al. 2013).

Protein Supply to Dairy Cows using the NRC-2001 model

The effect of canola seed variety on protein metabolic characteristics modeled with NRC-2001 is shown in Table 2. The CS_B was significantly lower than the CS_Y, in MCP_{TDN} and therefore lower in $AMCP^{NRC}$. Although no differences observed for the RUP^{NRC}, the dRUP was higher ($P < 0.05$) for the CS_Y compared to the CS_B. The DPB in our study was lower ($P < 0.05$) for the CS_B and positive for both types of canola seed which means that the availability of protein exceeds that one of energy for microbial protein synthesis. The degraded protein balance DPB shows the (im)-balance between

microbial protein synthesis from the available protein degraded in the rumen and from the potential energy from anaerobic fermentation in the rumen. In case that the DPB in a ration is positive, indicates a potential N-loss from the rumen and when is negative indicates a potential shortage of N in the rumen. So, the optimum degraded protein balance value in a ration is zero or slightly higher (Tamminga et al. 1994).

Feed milk value

The feed milk value (kg milk yield per kg feed) of both two canola seed varieties, predicted by NRC-2001 model and PDI system are shown in Table 3. The results showed no significant differences between the yellow and the black-seeded canola which is in accordance with the results reported by Xin et al. 2013 on B. carinata seed (yellow and brown type).

Comparison between the NRC-2001 Model and PDI System in Prediction of Protein Supply to Dairy Cows

The averages of the predicted values for CS_Y and CS_B modelled according to the NRC-2001 model and the PDI system, as well as the comparison among those models are presented in Table 4. The results showed that significant differences exist for all the nutrient supplies that predicted. However, the greatest differences were observed in the microbial protein supply, based on energy and in the truly absorbed rumen-synthesized microbial protein (-135 and -60.3 g/kg of DM, respectively). The same trend was observed on a study carried out on yellow and brown canola meal using the same evaluation systems (Theodoridou and Yu, 2013). Linear regression of the main average predicted nutritional values between the PDI system and the NRC-2001 model are presented in Table 5. The regression equations were significant ($P < 0.05$) for the microbial protein supply based on RUP ($R^2 = 0.99$), for the truly absorbed rumen-synthesised microbial protein ($R^2 = 0.97$) and the degraded protein balance ($R^2 = 0.95$) predicted according to the PDI system.

Conclusion

In conclusion, the PDI system predicted lower values for all the nutrient supply parameters studied compared to the NRC-2001 model. Independent of the feed evaluation system used in this study, both of them indicated that the microbial protein synthesis in the rumen based on the available protein and the rumen degraded protein absorbed in the small intestine, were lower for the yellow compared to the black canola seed. Given that the results reported in this study were outputs from mathematical models with data based on in vitro and in situ studies, it is very important to apply the predictions and evaluate them in animal trials.

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Table 1. Predicted values of potential nutrient supply to dairy cattle from yellow and black-type of canola seed (*B. Napus*) using the French PDI system

Item (g/kg of DM)	CS-Yellow *B. Napus* (n=2)	CS-Brown *B. Napus* (n=2)	SEM	P value
Absorbable microbial protein synthesis in the rumen (MPS^{PDI})[1]				
DOM	900.07	860.14	8.811	0.085
FOM	369.83	361.03	4.924	0.334
PDIME	33.50	34.32	0.457	0.332
PDIMN	83.35	99.77	2.083	0.031
MPS^{PDI}	26.83	33.41	0.613	0.017
Truly absorbable rumen un-degraded protein in small intestine (PDIA)[2]				
RUP^{PDI}	25.76	24.99	0.887	0.565
TId	90.42	85.99	0.575	0.032
PDIA	57.18	55.24	3.434	0.729
Degraded protein balance (DPB^{PDI})[3]				
PDIN (PDIN=PDIA+PDIMN)	140.52	155.01	5.506	0.204
PDIE (PDIE=PDIA+PDIME)	91.50	88.74	3.073	0.591
DPB^{PDI} (PDIN-PDIE)	49.02	66.27	2.495	0.039

[a - c] Means within a row with different superscripts differ ($P < 0.05$).
SEM = Standard error of mean.

Table 2. Predicted values of potential nutrient supply to dairy cattle from black canola seed and yellow canola seed (*B. Napus*) using the NRC-2001 model

Item (g/kg of DM)	CS-Yellow *B. Napus* (n=2)	CS-Black *B. Napus* (n=2)	SEM	*P* value
Absorbable microbial protein synthesis in the rumen ($AMCP^{NRC}$)[1]				
$MCP_{TDN}{}_{NRC}$	178.38	159.40	2.321	0.029
$MCP_{RDP}{}_{NRC}$	131.19	151.31	3.185	0.047
$AMCP^{NRC}$	83.96	96.84	2.035	0.047
Absorbable endogenous true protein in the small intestine (AECP)[2]				
ECP	11.22	11.16	0.013	0.093
AECP	4.49	4.47	0.005	0.106
Truly absorbable rumen un-degraded protein in small intestine ($ARUP^{NRC}$)[3]				
RUP^{NRC}	53.56	59.44	3.740	0.382
dRUP	56.59	44.56	2.786	0.022
$ARUP^{NRC}$	30.31	26.80	4.305	0.622
Total metabolisable protein (MP)[4]				
MP	118.76	128.10	5.962	0.383
Degraded protein balance (DPB^{NRC})[5]				
DPB^{NRC}	-56.15	-10.09	2.490	0.006

[a - c] Means within a row with different superscripts differ ($P < 0.05$).

[1]$MCP_{TDN}{}_{NRC}$ = microbial protein synthesized in the rumen based on available energy (discounted TDN); $MCP_{RDP}{}_{NRC}$ = microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; $AMCP^{NRC}$ = truly absorbed rumen synthesized microbial protein in the small intestine.

[2]ECP = rumen endogenous crude protein (CP); AECP = truly absorbed endogenous protein in the small intestine.

[3]RUP^{NRC} = ruminally undegraded feed CP, calculated according the formula in NRC-2001 dairy model; dRUP = intestinal digestibility of rumen undegraded crude protein, estimated according to Calsamiglia and Stern, 1995; $ARUP^{NRC}$ = truly absorbed rumen undegraded feed protein in the small intestine.

[4]MP = metabolizable protein (true protein that is digested postruminally and the component amino acid absorbed by the intestine) contributed by 1) ruminally undegraded feed CP, 2) ruminally synthesized microbial CP and 3) endogenous CP. (MP = AMCP + AECP + ARUP)

[5]DPB^{NRC} = reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

Table 3. Feed milk value of yellow and black canola seed (*B. Napus*) based on metabolic characteristics of protein predicted by NRC-2001 model and PDI system

Item (kg milk per kg feed)	CS-Yellow *B. Napus*	CS-Black *B. Napus*	SEM[1]	*P* value
Feed milk value using NRC-2011 system	2.41	2.60	0.121	0.384
Feed milk value using PDI system	2.85	3.15	0.112	0.204

[a - c] Means within a row with different superscripts differ ($P < 0.05$).

[1]SEM = Standard error of mean.

[2]The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (source NRC, 2001) and protein composition in milk is assumed 33 g protein per 1000 g milk.

Table 4. Comparison of the PDI system with NRC-2001 model in the prediction of protein supply to dairy cows from yellow and black-type of canola seed (*B. Napus*)

Item (g/kg of DM)	Mean PDI	Mean NRC 2001	Difference (PDI − NRC)[1]	SED[2]	P value	r^3	*P* value
Compared microbial protein supply based on available energy[4]							
PDIME vs MCP_{TDN}	33.9	168.9	-135.0	5.4750	0.0001	0.48	0.517
Compared microbial protein supply based on ruminally degraded feed protein[5]							
PDIMN vs $MCP_{RDP}{}^{NRC}$	91.6	141.2	-49.7	1.2620	<.0001	1.00	0.003
Compared truly absorbed rumen-synthesised microbial protein[6]							
MPS^{PDI} vs $AMCP^{NRC}$	30.1	90.4	-60.3	2.0276	<.0001	0.98	0.016
Compared truly absorbed rumen-undegraded feed protein[7]							
PDIA vs $ARUP^{NRC}$	56.2	28.6	27.7	1.2385	<.0001	0.90	0.103
Compared degraded protein balance[8]							
(PDIN-PDIE) DPB^{PDI} vs DPB^{NRC}	57.6	-33.1	24.5	8.3823	0.002	0.98	0.023

[1]Paired *t* test.
[2]Standard error of difference.
[3]Pearson correlation coefficient.
[4]PDIME: amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded nitrogen (N) is not limiting; MCP_{TDN}: microbial protein synthesized in the rumen based on available energy (discounted TDN).
[5]PDIMN: amount of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; $MCP_{RDP:}$ microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; AMCP: truly absorbed rumen synthesized microbial protein in the small intestine.
[6]MPS^{PDI}: truly absorbed rumen synthesized microbial protein in the small intestine; $AMCP^{NRC}$: truly absorbed rumen synthesized microbial protein in the small intestine.
[7]PDIA: dietary protein undegraded in the rumen, but truly digestible in the small intestine; $ARUP^{NRC:}$ truly absorbed rumen undegraded feed protein in the small intestine.
[8]DPB^{PDI}: balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen; DPB^{NRC}: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

Table 5. Regression equations for prediction of protein supply from PDI system based on values from NRC-2001 model for canola seed (*B. Napus*)

	Linear regression equation			
Item (g/kg of DM)	Equation y = a (±SE) + b (±SE) × x	R^2	*P* value	RSD[1]
Predicted microbial protein supply based on available energy[2]				
PDIME vs MCP_{TDN}	PDIME = 28.79 (±6.57) + 0.03 (±0.04) × MCP_{TDN}	0.23	0.517	0.76
Predicted microbial protein supply based on ruminally degraded feed protein[3]				
PDIMN vs MCP_{RDP}^{NRC}	PDIMN = -21.60 (±5.80) + 0.80(±0.04) × MCP_{RDP}^{NRC}	0.99	0.003	0.86
Predicted truly absorbed rumen-synthesised microbial protein[4]				
MPS^{PDI} vs $AMCP^{NRC}$	MPS^{PDI} = -13.95 (±5.64) + 0.49 (±0.06) × $AMCP^{NRC}$	0.97	0.016	0.84
Predicted truly absorbed rumen-undegraded feed protein[5]				
PDIA vs $ARUP^{NRC}$	PDIA = 36.57 (±6.95) + 0.69 (±0.24) × $ARUP^{NRC}$	0.80	0.103	2.23
Predicted degraded protein balance[6]				
(PDIN-PDIE) DPB^{PDI} vs DPB^{NRC}	DPB^{PDI} = 70.18 (±2.37) + 0.38 (±0.06) × DPB^{NRC}	0.95	0.023	2.71

[1]Residual standard deviation.

[2]PDIME = amount of microbial protein that could be synthesized from the available energy in the rumen, when degraded nitrogen (N) is not limiting; MCP_{TDN} = microbial protein synthesized in the rumen based on available energy (discounted TDN).

[3]PDIMN = amount of microbial protein that could be synthesized in the rumen from the degraded dietary N when energy is not limiting; MCP_{RDP} = microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; AMCP = truly absorbed rumen synthesized microbial protein in the small intestine.

[4]MPS^{PDI} = truly absorbed rumen synthesized microbial protein in the small intestine; $AMCP^{NRC}$ = truly absorbed rumen synthesized microbial protein in the small intestine.

[5]PDIA = dietary protein undegraded in the rumen, but truly digestible in the small intestine; $ARUP^{NRC}$ = truly absorbed rumen undegraded feed protein in the small intestine.

[6]DPB^{PDI} = balance between microbial protein synthesis from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen; DPB^{NRC} = reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen.

Project 6: EXPLORE PROTEIN MOLECULAR STRUCTURE IN ENDOSPERM TISSUES IN NEWLY DEVELOPED BLACK AND YELLOW TYPE CANOLA SEEDS BY USING SYNCHROTRON-BASED FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY (COMPLETED) *

A version of this project has been published: Katerina Thedoridou, Sally Vail, Peiqiang Yu. 2014. Explore Protein Molecular Structure in Endosperm Tissues in Newly Developed Black and Yellow Type Canola Seeds by Using Synchrotron-based Fourier Transform Infrared Microspectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (Elsevier). 120: 421–427 (DOI: 10.1016/j.saa.2013.10.034) (*as supervisor, PI, corresponding author).

Project Details

Abstract:

This study was conducted to characterize the protein molecular structure in endosperm tissues in newly developed black and yellow-type canola seeds by using synchrotron-based Fourier transform infrared microspectroscopy. The results showed that the yellow canola seeds contained relatively lower ($P < 0.05$) percentage of a-helix, β-sheet, amide I and amide II area compared to the black-type canola seed. This might be an indication that the protein value of the yellow canola seeds as food or feed is different from that of the black canola seeds. The multivariate molecular spectral analyses (AHCA, PCA) showed that there were not significant molecular structural differences in the protein amide I and amide II fingerprint region (ca 1720-1480 cm^{-1}) between the yellow and the black-type of canola seed. It can be concluded that both the yellow and the black-seeded canola contain the same proteins but in different ratios.

Keywords: Synchrotron microspectroscopy, Canola seed, Protein structure, Amide I,α-helix, β-sheet structures

Introduction

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in 1970's. Unlike with the traditional rapeseed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30 μmol in its defatted meal) [1]. The most common canola varieties used in Western Canada are the yellow-seeded and dark-brown-seeded varieties. It has been reported that yellow-seeded varieties are lower in fibre than brown-seeded types [2-4].

Research studies are mainly focused on total chemical composition of canola seeds including total protein, carbohydrates and dietary fibre components using the traditional "wet" chemical analysis [3-4]. The traditional analytical chemistry is currently employed to study a specific tissue component which is often separated it from the whole complex matrix. Consequently, the object prepared for analysis is usually destroyed and the information about the spatial origin and the component distribution is lost [5]. According to our knowledge, little research has been carried out to study the chemical bonding and functional groups of a feed seed tissue within cellular dimensions. It is supposed that these features are closely related to feed's quality and nutritive value for humans as well as for animals' diets.

One of the most important nutrients in animal and human diets is protein. Traditionally, when we determine protein quality in a feed or a food, we usually determine total protein or amino acid content. Accordingly, the resulting values are compared to a standard to determine the specific protein quality.

Due to the fact that, quality of protein relies not only on its content but also on its inherent structures (i.e. nutrient matrix and protein secondary structures), the method mentioned before appears to have a weakness [6]. Protein quality, nutrient utilization, availability or digestive behavior is associated with the access of gastrointestinal digestive enzymes to the protein, which is affected by the inherent protein structure [7-10]. The mostly occurring protein secondary structures include the α-helix and the β-sheet [11-13]. For the reasons mentioned, examine the protein secondary structure may be vital for understanding the digestive behavior, utilization and protein availability in both humans and animals [9].

Synchrotron-based vibrational FTIR microspectroscopy has been employed in order to reveal molecular structural features within the tissue in different kind of materials [14]. This technique is able to explore the molecular chemistry of biological samples with high signal-to-noise ratio at ultraspatial resolutions as fine as 3 to 10 μm due to the synchrotron light brightness [14-19]. This technique can be applied to detect information on ultrastructural chemistry by imaging or mapping without destruction of the intrinsic microstructures of plant tissue [20-21]. Research studies carried out previously, mentioned that using synchrotron-based FTIR microspectroscopy is feasible to i) compare plant tissue (i.e. species or varieties) according to spectroscopic characteristics, functional groups, spatial distribution and chemical intensity within cellular dimensions and ii) relate plant molecular structures to availability and utilization of plant tissue components [20-24].

Objective

The objective of this study was to use synchrotron light sourced FTIR microspectroscopy as an approach to define the molecular structural differences between the proteins of two newly developed canola seed varieties in the whole tissues at cellular and subcellular levels. Therefore is hypothesized that different types of canola seed (yellow, black) have different protein molecular structure profiles which may be detected by synchrotron infrared microspectroscopy.

Material and Methods

Feed samples

Yellow (Brassica Napus) and black (Brassica Napus) canola seeds were supplied by the *Agriculture and Agri-Food Canada*, Saskatoon Research Center, Canada and used as feed sources. Yellow and black canola seeds were collected from two different harvest years 2010 (total 4 kg) and 2011 (total 4 kg).
Synchrotron-based BaF2 window preparation

Seven seeds were randomly selected from each treatment (1=CS_Y10, 2=CS_Y11, 3=CS_B10 and 4=CS_B11) and then each seed was cut into thin cross sections (6 μm thickness per section; five sections per treatment) using a Microm 330microtome (Microm Laborgerate GmbH, Sandhausen, Germany) at the Western College of Veterinary Medicine, University of Saskatchewan. A more detailed methodology concerning the slide preparation was reported previously by Yu et al. [21], however here it will be briefly described. Unstained cross-sections were transferred to BaF2 windows (size: 13×1 mm disc; Spectral Systems, Hopewell Junction, NY, USA) for synchrotron-based microspectroscopic analysis in transmission mode. Photomicrographs of tissues cross-section were taken with a microscope linked to a digital camera from the BaF_2 window at U2b station in National Synchrotron Light Source at Brookhaven National Lab (NSLS-BNL, US Department of Energy, New York).

Synchrotron-based fourier transform infrared microspectroscopy data collection and analysis

Synchrotron experiment was carried out at the National Synchrotron Light Source in the Brookhaven National Laboratory (NSLS-BNL, US Department of Energy, NY). The detailed methodology has been reported previously [25] and the following is a brief explanation. The beamline U2B was equipped with a FTIR spectrometer (Magna-IR 860 Spectrometer, Nicolet Instruments, Madison, WI, USA) outfitted with a KBr beamsplitter and a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector coupled to a Nic-Plan IR microscope, a Schwartzshild 32×objective, and a 10×condenser. Synchrotron radiation from the Vacuum Ultraviolet storage ring (with an energy level of 800 MeV) entered the interferometer via a

port in the instrument designed for infrared emission. A range of 30-50 spot samples for each seed was randomly scanned in the relatively pure protein area in the endosperm region approximately 100-600 μm from the epidermis. The regions of high protein content were used in order to collect randomly the spectra within the mid-infrared range from 4000 to 800 cm^{-1} at a resolution of 4 cm^{-1} (128 co-added scans) and an aperture of 10_m×10_m. Scanned visible images were obtained by using a charge-coupled device camera connected to the infrared images.

Univariate and multivariate spectral data analyses

For the analysis of the spectral data obtained at the synchrotron-based Fourier transform infrared microspectroscopy, the univariate and multivariate approaches were adapted. The univariate method consists of various spot sampling and mapping displays of spectral data [26, 27]. On the other hand, the multivariate analysis method utilizes the entire spectral information and creates spectral corrections. The agglomerative hierarchical cluster analysis (AHCA) which uses the Ward's algorithm method without prior parameterization, and the principal component analysis (PCA) are encompassed in the multivariate method.

Univariate molecular spectral analysis of protein structure profiles

The spectrum collection, the corrections with the background spectrum, the classification of protein's structure functional spectral bands and the data analysis was carried out using the software program OMNIC 7.3 (Spectra Tech, Madison, WI, USA).

Protein amide I and II peak area intensities as well as absorption peak heights for secondary protein structure α-helices (ca. 1660 cm^{-1}) and β-sheets (ca. 1630 cm^{-1}) are detectable in the amide I area using the second derivative function of OMNIC 7.3 (Spectra Tech, Madison, WI) and assigned according to published studies [27-31]. Unique primary protein features found in peptide bonds (C-O, C-N and N-H) include amide I (~80% C=O and ~20% C-N stretching vibration; centered at a wavelength of ca. 1655 cm^{-1}) and amide II (~60% N-H bending vibration,~40% C-N stretching vibration; ca. 1550 cm^{-1}) which are detectable as two absorption peaks within the wavelength region from ca. 1720 to 1485 cm^{-1} as described previously in other research studies [32-36]. The α-helix to β-sheet spectral intensities and the ratios of amide I to amide II were calculated.

Multivariate molecular spectral analysis of protein structure profiles

The spectra comparison and distinguish of the inherent structural differences between the yellow-type and the black-type canola seed was performed by using a multivariate analysis. Agglomerative hierarchical cluster analysis results were displayed as dendrograms and PCA results were plotted based on the two highest factor scores and presented as a function of those scores. During all the comparisons that carried out the eigenvector for factor 1 was plotted against that for factor 2 which accounted for over 99% of the data variability. These analyses were carried out using Statistica 8 software (StatSoft Inc., Tulsa, OK, USA).

Statistical analysis

The statistical analysis for the synchrotron-based protein structure study was performed using the PROC MIXED of SAS using a completed nested design. For the analysis the following model was used:

$$Yij = \mu + Ti + S(T)j + eij,$$

where Yij was an observation of the dependent variable ij (amide I, amide II, ratio of amide I to amide II, α-helix, β-sheet or ratio of α-helix to β-sheet); μ was the population mean for the variable; Ti was the effect of the variety as a fixed effect; S (T)j is the seeds nested within the variety, as a random effect; and eij was the random error associated with the observation ij. The detailed methodology was reported in a previously research study [25].

Results and Discussion

Protein molecular structure spectral profiles of yellow and black canola seeds

The protein molecular structure profiles of yellow and black type canola seeds defined with synchrotron-based FTIR microspectroscop*y* are presented in Table 1. Protein structure profile influences protein value (e.g. absorbed protein in small intestine) and functionality (e.g. solubility) and affects the access of gastrointestinal digestive enzymes to the protein. Reduced accessibility results in poor digestibility and as a result, low-protein nutritive value (44). For those reasons the information obtained from secondary protein structure helps to understand the digestive fate and nutritive value in animals. Amide I and II bands are linked to different functional groups in proteins, which can be measured quantitatively in terms of peak area or height (7). The ratio of amide I:amide II describes differences in the overall molecular structures of proteins and varies between biological tissues (8). In our study the amide I and II areas values were higher (P <0.05) for black-seeded canola compared to those for yellow-seeded one while no differences observed in amide I to amide II height.

In terms of β-sheet height, the highest level was found for black canola seed. The relative proportions of the main features of secondary protein structures, α-helices and β-sheets influence protein nutritive value, quality and digestive fate (7). High percentage of β -sheet structure may partly cause low access to gastrointestinal digestive enzymes, which results in a low protein value (7). In case that the ratios of α-helices and β-sheets differ in the protein inherent structure, then consequently the protein nutritive value may also differ (10, 7, 25).

The main protein fractions in rapeseed protein are those of cruciferin and napin as have been shown previously in the SDS polyacrylamide gel electrophoresis (PAGE) profiles [37]. The last mentioned protein fractions represent the storage proteins for *Brassica* seeds and are responsible for the functional and nutritive quality of the total rapeseed protein [38-39]. The α-helical structure content of napin fraction is about 40 to 46% while appears a low content of β-sheet conformation (12%) [40]. On the other hand cruciferin, has low content of α-helical structure (10%) and a high content of β-sheet conformation (50%) [41]. In this study the higher β-sheet value that obtained for black canola meal may be an indication of a higher cruciferin protein fraction in this canola seed variety.

Results of the present research work showed that SR-FTIRM can be employed in order to reveal molecular functional groups at ultra-spatial resolution [14]. The SR-FTIRM provides the operator with an accurate and precise study of a limited seed section profile, of a fast observation recordings acquisition, reaches diffraction limit as a few μm and delivers a sufficient signal to noise ratio at ultra-spatial resolutions [16,27,19,35,36]. By using the SR-FTIRM the biological tissue prepared for examination is not destroyed and so the feed structural–chemical features within cellular dimensions could be investigated.

Multivariate analysis to discern inherent molecular structures in yellow and black canola seed

Discrepancies in molecular structure and functional groups have to be explored and for that reason multivariate spectral analyses are applied. One of the multivariate analyses is the cluster one, which performs an analysis of an infrared spectra data set and lays out the results as a dendrogram. In the present study, the Ward's algorithm method was applied without any prior parametrization of the spectral data in the IR region of protein amides. The success to apply multivariate AHCA analysis has been reported previously [42] for the three feed inherent structures (structure 1, feed pericarp; structure 2, feed aleurone; structure 3, feed endosperm) and different varieties of canola. Theodoridou and Yu [43] for different varieties of canola meal and presscake, Doiron et al.[44] for heat treated flaxseed and Liu and Yu[45] for various barley genotypes. Another multivariate analysis is the principal component analysis which transforms the original variable set to a new one, called principal components. The first few principal components will attribute to a high percentage variance (>95% variance). The PCA analysis presents the results as scatterplots among the components. The purpose of this kind of analysis is to obtain limited independent linear combinations of a set of variables that retain as much of the information in the original variables as possible.

Therefore, PCA allows the feed intrinsic structures to be distinguished and identifies features that differ among feed structures. Figure 1 shows typical spectra, smoothed spectra and 2nd derivative spectra

of yellow and black canola seed revealed by synchrotron-based FTIR microspectroscopy. It is hard to visually detect the spectral difference between the yellow and black canola seed.

The agglomerative hierarchical cluster and the principal component analysis were applied to investigate the differences in a molecular structure level between the yellow and the black-type canola seeds. The PCA analyses of molecular spectral (amide I and amide II region: *ca.* 1720-1480 cm^{-1}) obtained from the canola seed samples (Figure 2), indicated that there were no significantly molecular structural differences in the amide I and II region as they did not form two separate classes and were not groped in separate ellipses. As it was showed from the spectrum of yellow and black-seeded canola, the scatterplot of the first principal component explained 69.35% of the total variance whiles the second principal component 28.27%

The mixed dendrogram of yellow and black canola seed showed a similarity of spectral data in their amide I and II regions, indicating that these two feedstuffs were not completely different in protein spectroscopic features (Figure 2).

Conclusion

From the present research study, it was deduced that the black-type canola seed comprises a higher percentage of a-helix, β-sheet, amide I and amide II area in comparison to the yellow-type canola seed. The results obtained from the agglomerative hierarchical cluster analysis and the principal component analysis showed that there were not significant molecular structural differences, concerning the protein amide I and II fingerprint region, between the yellow and black type of canola seed. The application of synchrotron-based Fourier transform infrared microspectroscopy along with the univariate and multivariate analyses is capable to localize relatively "pure" protein in different plant tissues and explore their protein molecular structure. This can be conducted, without any wreckage of the native structure of the tissue (i.e. canola seed), and gives an indication of its nutritive value. All the information related to the nutritive value of seeds, could be used in the future by breeders in different breeding programs for picking of varieties intended for human and animal consumption.

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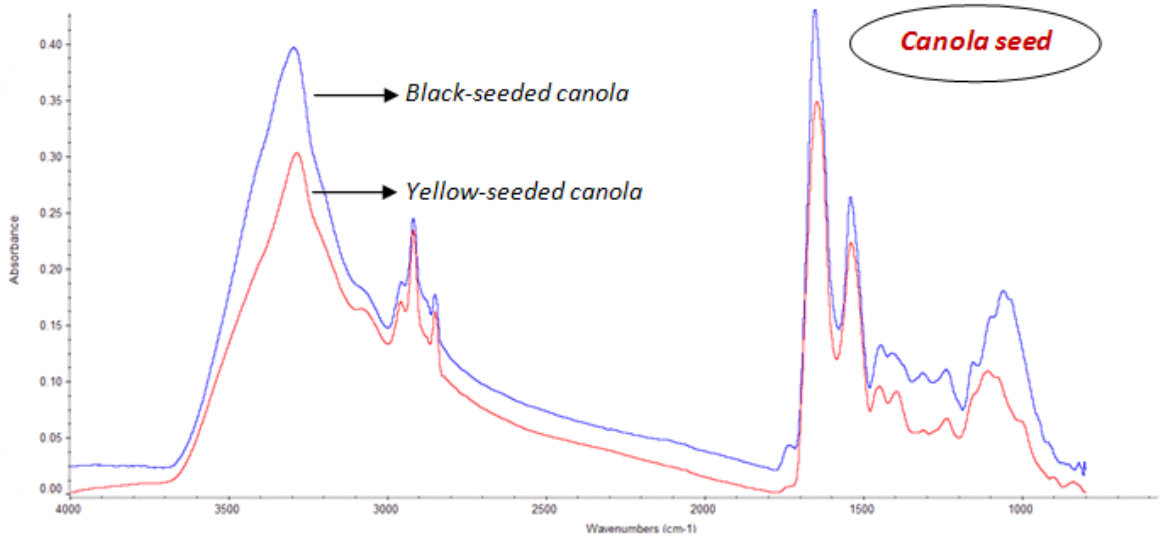
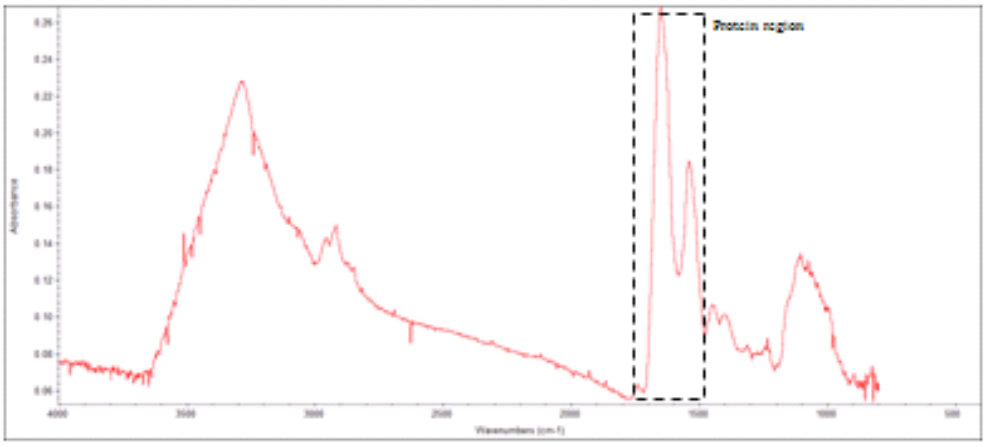
Table 1.
Comparison between yellow (CS_Y) and black (CS_B) canola seeds at endosperm region in terms of protein molecular structure spectral profiles using synchrotron-based Fourier transform infrared microspectroscopy.

	Endosperm region			
Item	CS_Y	CS_B	SEM	*P* value
Protein molecular structure spectra profiles (Unit: Absorbance)				
Amide I area	14.675[b]	17.775[a]	1.0649	0.0497
Amide II area	6.176[b]	7.549[a]	0.4361	0.0349
Ratio amide I to amide II area	2.380[a]	2.356[a]	0.0196	0.4188
Amide I height	0.204[a]	0.247[a]	0.0154	0.0598
Amide II height	0.104[a]	0.125[a]	0.0074	0.0571
Ratio amide I to amide II height	1.965[a]	1.989[a]	0.0130	0.1900
Protein secondary structure profile				
a-helix (height)	0.202[a]	0.246[a]	0.0155	0.0541
β-sheet (height)	0.170[b]	0.206[a]	0.0117	0.0401
Ratio a-helix to β-sheet	1.184[a]	1.194[a]	0.0203	0.7370

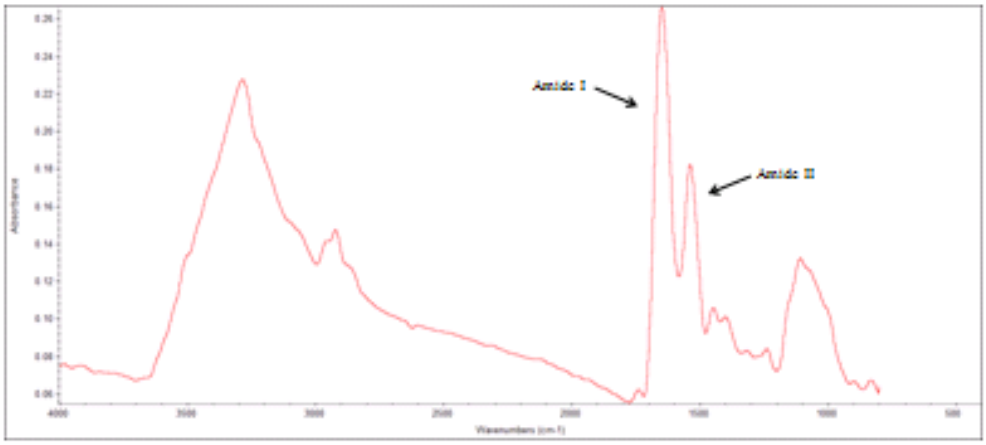
SEM= Standard error of mean; [a, b, c] Means with different letters within the same row differ ($P < 0.05$).

TOC Graphic

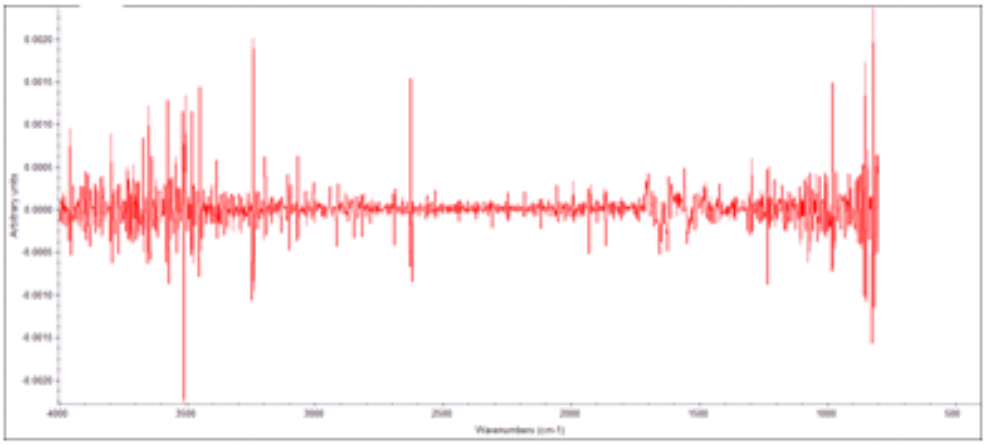

Canola seed
Black-seeded canola
Yellow-seeded canola
Absorbance
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
4000
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)



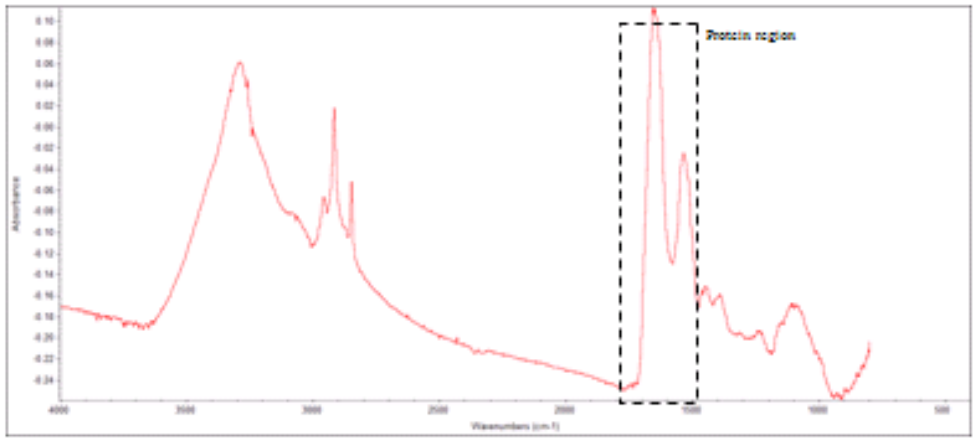
A: Yellow canola seed (Original spectra)



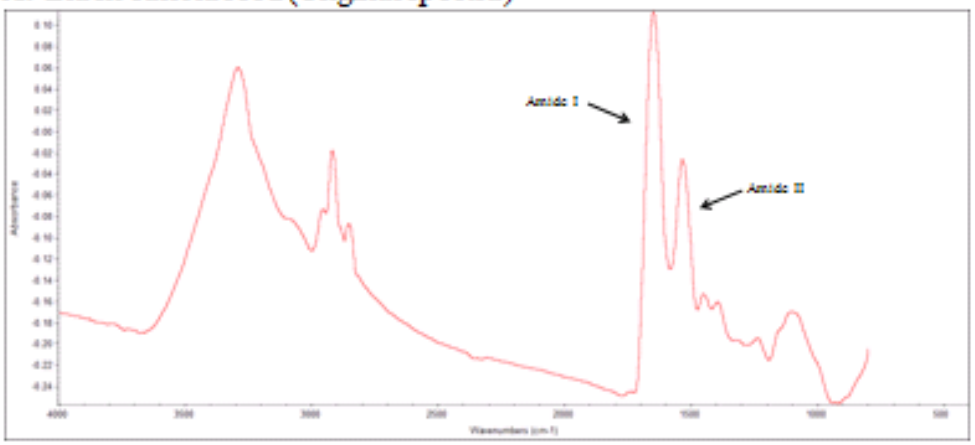
B: Yellow canola seed (Smoothed spectra)



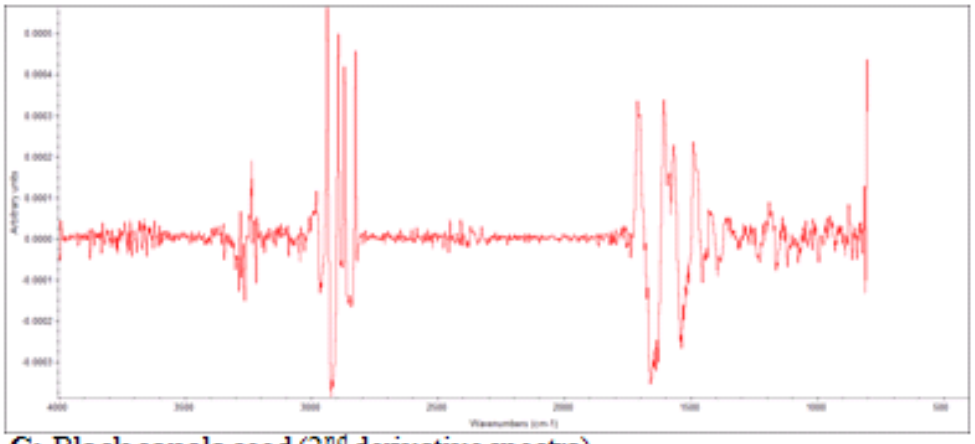
C: Yellow canola seed (2nd derivative spectra)



A: Black canola seed (Original spectra)

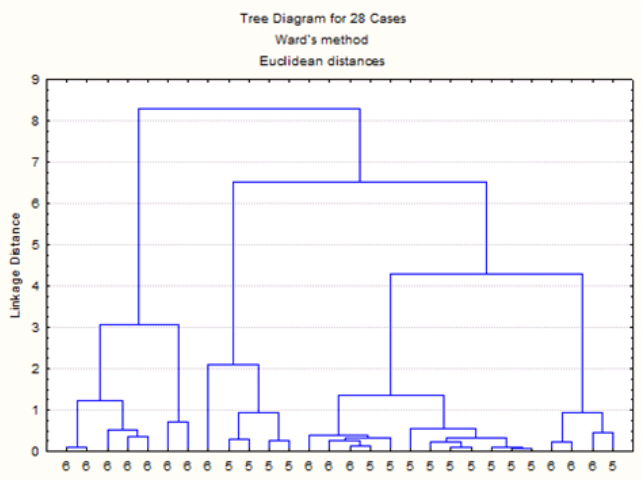


B: Black canola seed (Smoothed spectra)

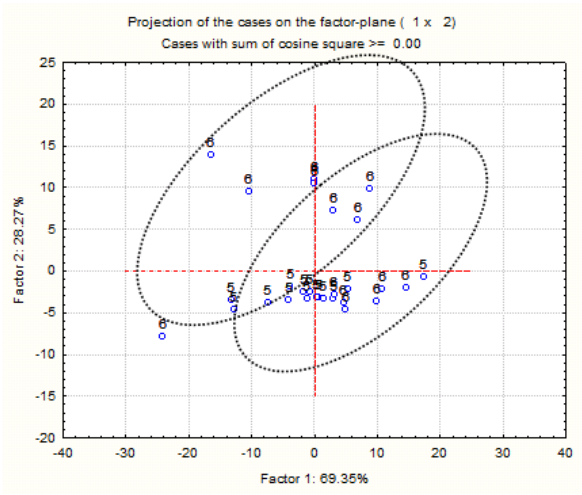


C: Black canola seed (2nd derivative spectra)

Figure 1. Typical spectra (original spectra, smoothed spectra and 2nd derivative spectra) of yellow and black canola seed.



I: 5 = CS_Y *vs* 6 = CS_B
Cluster (CLA) spectral analysis: (1) select spectral region: amide I and amide II regions (*ca* 1720-1480 cm^{-1}); (2) distance method: Euclidean; (3) cluster method: Ward's algorithm.



I: 5 = CS_Y *vs* 6 = CS_B
Scatter plot of the 1st principal component *vs* the2nd principal component. The 1st and 2nd principal component explains 69.35% and 28.27% of the total variance respectively.

Figure 2. Multivariate molecular spectral analyses of amide I and amide II regions of the feedstuffs used in this study: (I) comparison of yellow canola seed (CS_Y) and black canola seed (CS_B).

Program III:

Graduate Reseach Thesis Projects

This Program provide two graduate student training and the following are two graduate thesis.

Project 7: 1st MSC THESIS PROJECT: IMPROVEMENTS IN NUTRITIVE VALUE OF CANOLA MEAL WITH PELLETING (COMPLETED) *

*A version of part of this project has been published:

X. Huang, N. A. Khan, X. Zhang, P. Yu*. 2015. Effects of Canola Meal Pellet Conditioning Temperature and Time on Ruminal and Intestinal Digestion, Hourly Effective Degradation Ratio, and Potential N to Energy Synchronization in Dairy Cows. Journal of Dairy Science (USA). 98: 8836–8845 (DOI: 10.3168/jds.2014-9295) (*as Supervisor, PI and Corresponding Author).

X. Huang, C. Christensen, P. Yu*. 2015. Effects of Conditioning Temperature and Time during the Pelleting Process on Feed Molecular Structure, Pellet Durability Index, Metabolic Features of Co-Products from Bio-Oil Processing in Dairy Cows. Journal of Dairy Science (USA). 98: 4869-4881 (DOI:10.3168/jds.2014-9290) (*as Supervisor, PI and Corresponding Author.)

Please see the enclosed for the Full Graduate Thesis.

Project 8: 2nd MSC THESIS PROJECT: STRUCTURAL, PHYSIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF NEWLY DEVELOPED CANOLA SEEDS and BRASSICA CARINATA AND THE CO-PRODUCTS (COMPLETED) *

Please see the enclosed for Full Graduate Thesis.

Highly Qualified Personal Training from This Research Program:

- PDF Training: PDF fellows involved: Katerina Theodoridou was nvolved in this feed research program.
- Two MSc Graduate Students Training: Two MSc students (Ms. Yuguang Ying and Mr. Xuewei Huang);
- One joint training PhD student (Xinxin Li) was involved in this feed research program.

Technology Transfer, Extension Activities, Publications, and Industry Presentations/ Seminars from This Research Program

Three Research Thesis –Three Graduate Students:

- **1st Graduate Thesis**: Xuewei Huang. 2015. IMPROVEMENTS IN NUTRITIVE VALUE OF CANOLA MEAL WITH PELLETING. MSc Thesis. University of Saskatchewan, Canada (Thesis Defence was suceffully done in Feb 10, 2015)
- **2nd Graduate Thesis**: Yajing Ban. 2015. STRUCTURAL, PHYSIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF NEW CANOLA SEEDS AND NEW BRASSICA CARINATA AND THE CO-PRODUCTS. MSc Thesis. University of Saskatchewan, Canada (Permission to write was granted on Sept 29, 2015; Draft thesis ready in Feb 2016)
- **3rd Graduate Thesis**: Xinxin Li. 2016. FEED MILK VALUES FOR DAIRY CATTLE: CANOLA MEAL VS. CHINESE RAPESEED MEAL VS. SOYBEAN MEAl. PhD Project. Northest Agricultural University, China and University of Saskatchewan, Canada (Thesis writing is ongoing)

Publications of Research Findings in Peer-Reviewed Journals: (Note: *as supervisor, PI, corresponding author).

- X. Huang, N. A. Khan, X. Zhang**,** P. Yu*. 2015. Effects of Canola Meal Pellet Conditioning Temperature and Time on Ruminal and Intestinal Digestion, Hourly Effective Degradation Ratio, and Potential N to Energy Synchronization in Dairy Cows. Journal of Dairy Science (USA). 98: 8836–8845 (DOI: 10.3168/jds.2014-9295) (*as Supervisor, PI and Corresponding Author).
- X. Huang, C. Christensen, P. Yu*. 2015. Effects of Conditioning Temperature and Time during the Pelleting Process on Feed Molecular Structure, Pellet Durability Index, Metabolic Features of Co-Products from Bio-Oil Processing in Dairy Cows. Journal of Dairy Science (USA). 98: 4869-4881 (DOI:10.3168/jds.2014-9290) **(***as Supervisor, PI and Corresponding Author.**)**
- K. Thedoridou, X. Zhang, S. Vail, P. Yu*. 2015. Magnitude Differences in Bioactive Compounds, Chemical Functional Groups, Fatty Acid Profiles, Nutrient Degradation and Digestion, Molecular Structure, and Metabolic Characteristics of Protein in Newly Developed Yellow-Seeded and Black-Seeded Canola Lines. J. Agric. Food Chem (USA). 2015, 63: 5476–5484 (DOI: 10.1021/acs.jafc.5b01577) **(***as Supervisor, PI and Corresponding Author**)**.
- Katerina Theodoridou and Peiqiang Yu*. 2016. Protein and energy metabolic characteristics and nutrient supply, to ruminants, from newly developed canola lines, as predicted using the NRC-2001 model and the PDI system. Manuscript Draft is Ready (*as supervisor, PI, corresponding author).
- Katerina Theodoridou and Peiqiang Yu*. 2013. Effect of Processing Conditions on the Nutritive Value of Canola Meal and Canola Presscake: Comparison of the Yellow-Seeded (Brassica Juncea) and the Brown-Seeded (Brassica Napus) Canola Meal with the Brown-Seeded (Brassica Napus) Canola Presscake. J. Sci. Food Agric (England, UK). 93:1986-95 (DOI: 10.1002/jsfa.6004) (*as supervisor, PI, corresponding author).
- Katerina Theodoridou and Peiqiang Yu*. 2013. Metabolic Characteristics of the Proteins in

Yellow-Seeded and Brown-Seeded Canola Meal and PressCake in Dairy Cattle: Comparison of Three Systems (PDI, DVE, NRC) in Nutrient Supply and Feed Milk Value (FMV). J. Agric. Food Chem (USA). 61: 2820−2830 (DOI: 10.1021/jf305171z) (*as supervisor, PI, corresponding author).

- Katerina Theodoridou and Peiqiang Yu*. 2013. Application potential of ATR-FT/IR molecular spectroscopy in animal nutrition: Reveal protein molecular structures of canola meal and presscake, as affected by heat processing methods, in relationship with their protein digestive behavior and utilization for dairy cattle. J. Agric. Food Chem (USA). 61: 5449−5458 (DOI: 10.1021/jf400301y) (*as supervisor, PI, corresponding author).
- Katerina Thedoridou, Sally Vail, Peiqiang Yu*. 2014. Explore Protein Molecular Structure in Endosperm Tissues in Newly Developed Black and Yellow Type Canola Seeds by Using Synchrotron-based Fourier Transform Infrared Microspectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (Elsevier). 120: 421–427 (DOI: 10.1016/j.saa.2013.10.034) (*as supervisor, PI, corresponding author).
- X. Zhang, P. Yu*. 2014 Using Non-Invasive Technique in Nutrition: SR-IMS Spectroscopic Characterization of Oil Seeds Treated with Different Processing Conditions on Molecular Spectral Factors Influencing Nutrient Delivery. J. Agric. Food Chem (USA). 62: 6199–6205 (DOI: 10.1021/jf501553g) **(***as Supervisor, PI and Corresponding Author)
- Peiqiang Yu*, Katerina Theodoridou, Hangshu Xin, Pei-Yu Huang, Yao-Chang Lee, Bayden R. Woods. 2013. Synchrotron-Based Microspectrsopic Study on the Effect of Heat Treatment on Cotyleton Tissues in Yellow-Type of Canola (Brassica) Seeds. J. Agric. Food Chem (USA). 61: 7234–7241. DOI: 10.1021/jf4012517)
- X. Huang, P. Yu*. 2016. Investigation of Structure Interaction to Nutrient Properties and Utilization in Co-Products after Pellet Processing at Various Conditions Using Advanced Molecular Spectroscopy. Applied Spectroscopy Reviews. In press (DOI: 10.1080/05704928. 2016.1152975) **(***as Supervisor, PI, Corresponding Author).

Research Book Chapters:

- Xuewei Huang, Peiqiang Yu*. 2015. Book Chapter 5. Physical and Chemical Characterization, Technological Processing, Utilization and Benefit: Oilseed and Co-Products from Bio-Oil Processing, In: Advances in Chemistry Research, Vol. 29: In press, ISBN 978-1-63483-594-7; Editor: James C. Taylor; Nova Science Publishers, Inc. New York. USA.

CTV News- Extension Activities for This Project:

CTV News, My PDF Katerina Theodoridou, SaskCanola NSERC-CRD funded canola research program was Interviewed by Bob Simpson - CTV Saskatchewan Farm News Director on May 28, CTV News about our canola meal and seed research program from 29-31 May 2014.

Industry Magazine Interview-Extension Activities from My Team

Industry Magazine: "**The Western Producer**" - Canada's Best Source for Agriculture News and Information: Interviewed my PDF, K. Theodoridou, about our SaskCanola NSERC-CRD funded canola research program by William DeKay from "**The Western Producer**" on 17Mar2014.
The article title: "Saskatchewan Researchers Explore Canola Seed as Feed - Analyzing Meals and Cakes | Nutrition Research Reveals Yellow-Seeded Canola Meal Could be an Effective Alternative Animal Protein". The Western Producer, V92. No 13. Mar 27, 2014, p 51. Article available online: http://www.producer.com/issue/the-western-producer-march-27-2014/

Tech Transfer/Extension Activities: Articles for Industry Magazine and Newsletter:

- In: "The Milk Producer (National Canadian Dairy Magazine)", titled "K. Theodoridou and Peiqiang Yu* 2014. APPLIED SCIENCE: Distinguishing Feed Value: Researchers' Study the Differences between Canola Meal and Canola Presscake in Dairy Cattle Feed. The Milk Producer, Vol. 89, No. 11, pp 32-33. Available Online: http://www.milk.org/corporate/

view.aspx?content=aboutus/MilkProducerMagazine (*Role: as supervisor, PI, and corresponding author).

Tech Transfer /Extension Activities: Presentations/Talks in Professional and Industry Meetings (*as PI, supervisor, corresponding author).

Presentations at Various Industry Meetings:

- Presentation for "33rd Western Canadian Dairy Seminar in 2015": Yajing. Ban, Dave A. Christensen, John J. McKinnon, Peiqiang Yu*. 2015. Chemical Profiles, Energy Values, Protein and Carbohydrate Fractions of New Co-products (Carinata Meal) from Bio-fuel Processing as a New Alternative Feed for Dairy Cattle in Comparison with Canola Meal. Advances in Dairy Technology. Vol 27, pp 359 (The abstract was selected as one of four graduate students for oral student presentation competition. Yajing Ban won the 3rd place in the competition in the 33rd Western Canadian Dairy Seminar). Proceedings of the 2015, the 33rd Western Canadian Dairy Seminar, Lorraine Doepel, Editor, Published byUniversity of Alberta, Edmonton, Alberta, Canada.
- Presentation for "Soil and Crops 2015": Katerina Theodoridou, and Peiqiang Yu* 2015. Explore Protein Molecular Structure and Nutritive Value of Yellow and Black Canola Seed. Soils & Crops 2015, 16-17 March, Saskatoon, Canada (*as supervisor, PI, corresponding author).
- Presentation to Dairy Producers for "Third Annual Dairy Info Day", Brian King Centre, Warman SK, Jan 30, 2014, titled "K. Theodoridou and **Peiqiang Yu***. 2014. Feed Milk Value (FMV) and Metabolic Characteristics of the Proteins in Yellow-Seeded and Brown-Seeded Canola Meal and Presscake in Dairy Cattle." Proceedings of the Third Annual Dairy Info Day, Brian King Centre, Warman SK, Jan 30, pp 7-8 (*Role: as supervisor, PI, and corresponding author).
- Presentation 1 for "Soil and Crops 2014", X. Huang, T. Scott, C. Christensen, F. Buchanan and **Peiqiang Yu*** 2014. Effect of pelleting process at different conditions on in situ rumen degradation kinetics and N to energy synchronizations of canola meals in dairy cattle. Soils & Crops 2014, 11-12 March 2014, Saskatoon, Canada.
- Presentation to Saskatchewan Producers for "Soils and Crops 2014", Saskatoon, Canada, 11-12 March 2014, titled "Katerina Theodoridou and **Peiqiang Yu*** 2014. Evaluation of the feed value for ruminants of newly developed black and yellow type of canola seeds". Soils & Crops 2014, 11-12 March 2014, Saskatoon, Canada (*Role: as supervisor, PI, and corresponding author).
- Presentation for "Soil and Crops 2013" and written "**Industry Article** (pp-1-5)"- SaskCanola and NSERC-CRD Canola Research Program: Katerina Theodoridou, Peiqiang Yu*. Detect protein molecular structure of canola meal and presscake due to processing conditions, in relation with their protein digestive behavior and nutritive value. Soils & Crops Conference 2013. Saskatoon, SK, Canada.
- Presentation for Feed and Animal Nutrition Industry "**Western Nutrition Conference**" - SaskCanola and NSERC-CRD Canola Research Program: Katerina Theodoridou, **Peiqiang Yu*. 2013.** Effect of processing conditions on the nutritive value of canola meal and canola presscake. Comparison of the yellow (Brassica juncea) and the brown-seeded (Brassica napus) canola meal with the brown-seeded (Brassica napus) canola presscake. e-Proceeding of the 34th Western Nutrition Conference- Processing, Performance & Profit, Saskatoon, Canada, September 24-26, 2013, **pp 196.**
- Presentation for Feed and Animal Nutrition Industry "**Western Nutrition Conference**" - SaskCanola and NSERC-CRD Canola Research Program: Xuewei Huang, Tom Scott, Colleen Christensen, Fiona Buchanan, **Peiqiang Yu*. 2013.** Effect of pelleting process at different conditions on chemical and nutrient profiles of canola meals. e-Proceeding of the 34th Western Nutrition Conference- Processing, Performance & Profit, Saskatoon, Canada, September 24-26, 2013, **pp 184.**
- **Research Display** - SaskCanola and NSERC-CRD Canola Research Program **on Opening Day of the New Dairy Teaching and Research Facility, 17Oct2013 "**Katerina Theodoridou, **Peiqiang Yu*. 2013.** Effect of processing conditions on the nutritive value of canola meal and canola

presscake. Comparison of the yellow (Brassica juncea) and the brown-seeded (Brassica napus) canola meal with the brown-seeded (Brassica napus) canola presscake".

- **Research Display** - SaskCanola and NSERC-CRD Canola Research Program **on Opening Day opening day of the new Dairy Teaching and Research Facility, 17Oct13: "**Xuewei Huang, Tom Scott, Colleen Christensen, Fiona Buchanan, **Peiqiang Yu*. 2013.** Effect of pelleting process at different conditions on chemical and nutrient profiles of canola meals."

Presentations at Professional Meetings: **ADSA-ASAS-CSAS Joint Annual Meeting (**ADSA= American Dairy Science Society; ASAS= American Society of Animal Science; CSAS= Canadian Society of Animal Science**).**

- Yajing Ban, David A. Christensen, John J. McKinnon, and Peiqiang Yu*. 2015. Metabolic characteristics of protein, nutrient supply and feed milk value of the newly developed AAFC yellow and brown carinata lines for dairy cattle in comparison with commercial canola seeds. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 56. **(**Graduate Student Competition: ADSA Production Division Graduate Student Poster Competition, MS) (*****My role: as supervisor, PI, corresponding author)
- Yajing Ban, David A. Christensen, John J. McKinnon, and Peiqiang Yu*. 2015. Protein chemical profile, energy values, protein fractions, and rumen degradation characteristics of the newly developed yellow and brown carinata lines for dairy cattle compared with commercial canola seeds. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 308. **(***as supervisor, PI, corresponding author)
- Yajing Ban, David A. Christensen, John J. McKinnon, and Peiqiang Yu*. 2015. Feed milk value and protein supply to dairy cows of new co-products (carinata meal) from bio-fuel processing in comparison with canola meal. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 138 (*as supervisor, PI, corresponding author)
- Xuewei Huang, Tom Scott, Colleen Christensen, Yajing Ban, Xinxin Li and Peiqiang Yu*. 2015. Pelleting-induced changes at different conditioning temperatures and times on metabolic characteristics of the proteins and feed milk value of co-products from bio-oil processing.. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 166. **(*** as supervisor, PI, corresponding author)
- Xuewei Zhang, Limei Chen, Yajing Ban, and Peiqiang Yu*. 2015. Detect the association of protein structures to protein nutrient utilization and availability of co-products from bio-fuel and bio-brewing processing. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 479. (*as supervisor, PI, corresponding author)
- Katerina Theodoridou, Yajing Ban and Peiqiang Yu*. 2015. Protein molecular structure and nutritive value of yellow and black canola seed. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2: 774. (*as supervisor, PI, corresponding author)
- Xuewei Huang, P. Yu*. 2014. Effect of pelleting at different conditions on ruminal degradation kinetics and intestinal digestion of canola meal in dairy cattle. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 106. (*as supervisor, PI, corresponding author).
- K. Theodoridou, P. Yu*, H. Xin and X. Huang. 2014. Feed value for ruminants of newly developed black and yellow type of canola seeds. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 835. (*as supervisor, PI, corresponding author)
- Xuewei Huang, Peiqiang Yu*. 2014. Effect of pelleting at different conditions on ruminal degradation kinetics and intestinal digestion of canola meal in dairy cattle. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 106. (* as graduate student supervisor) (2014 Joint Annual Meeting Abstract Book, 2014 ADSA-ASAS-CSAS Joint Annual Meeting, July 20-24, 2014)
- K. Theodoridou, Peiqiang Yu*, H. Xin and X. Huang. 2014. Feed value for ruminants of newly developed black and yellow type of canola seeds. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 835. (* as supervisor)
- Yu, P. 2012. The Synchrotron (SR-IMS) as a Research Tool for Plant, Seed and Feed Science Research. 2012-The Joint National Synchrotron Light Source and Center for Functional

Nanomaterials (NSLS/CFN) Users' Meeting, Brookhaven National Lab, U.S. Dept. of Energy, USA, May 21-23, 2012 (Invited Presentation).

- Theodoridou K and Peiqiang Yu*. 2012. Changes of protein molecular structure of canola meal and canola press-cake due to heat processing and variety in relation to their nutritive value. Proceeding of the 15th Canadian Light Source Annual Users' Meeting Program. May 3-4, 2012, Saskatoon, Canada, pp 79