EFFECT OF GENOTYPES, TANNIN LEVEL AND PROCESSING METHODS ON THE PHYSICOCHEMICAL, NUTRITIONAL AND STRUCTURAL CHARACTERISTICS OF FABA BEAN GROWN IN WESTERN CANADA.

A Thesis Submitted to the

College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

By

María Eugenia Rodríguez Espinosa

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Master of Science degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes, may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan for any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Dean of College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan S7N 5C9

Canada.

Dean of the Department of Animal and Poultry Science

University of Saskatchewan

2D30 Agriculture Building, 51 Campus Drive

Saskatoon, Saskatchewan S7N 5A8

Canada.

ABSTRACT

The general objective of this study was to determine the effect of genotypes, tannin level (low and normal) and heat processing methods on the structural, physicochemical, and nutritional characteristics of faba bean grown in western Canada as an alternative protein and energy source for ruminants. The faba bean used for this research were grown in three different locations in Saskatchewan and included three low tannin types (LT): Snowbird, CDC Snowdrop, CDC 219_16 and five normal tannin types (NT): CDC Fatima, Vertigo, 346_10, FB9_4, and CDC SSNS_1. Chemical analyses, energy parameters, rumen degradation kinetics, intestinal digestion and FTIR molecular spectroscopic analyses were presented in this research. Statistical analyses were performed using the MIXED procedure model of SAS 9.4 with RCBD in Study 1 (genotypes as a fixed effect and locations as a random block effect). CRD was used in Study 2 (with 2 x 3 factorial design including two tannin levels and three treatments (Control, Steam Pressure and Microwave Irradiation). Significance in all studies was declared at $P < 0.05$ with tendency at $P \leq 0.10$. In Study 1, the results of chemical profiles showed no significant differences in total protein (CP) and total carbohydrates (CHO) contents among genotypes. Low tannin types of faba bean showed higher ($P < 0.05$) soluble crude protein (SCP), rumen bypass starch (BSt), and intestinal digestibility of rumen bypass protein (dIDP) compared to normal tannin. On the other hand, NT faba bean had greater organic matter (OM), lignin (ADL), slowly degradable protein fraction (PB2), and total tract digested starch (TDST) compared to low tannin. No significant difference ($P > 0.10$) was observed on total digestible nutrients (TDN_{1x}), metabolizable protein (MP), rumen degraded protein balance (DPB), feed milk value (FMV), and rumen undegraded crude protein (RUP). In Study 2 (Heat Processing), Steam Pressure (SP) and Microwave Irradiation (MI) increased ($P < 0.05$) the indigestible crude protein (PC) and decreased the rapidly degradable CHO fraction

(CA4), indigestible fiber fraction (CC), and undigestible neutral detergent fiber ($uNDF_{288h}$). Steam Pressure reduced ($P < 0.05$) the digestibility of bypass starch (dBST) and total digested starch (% TDST) and increased ($P < 0.05$) the intestinal digested crude protein (IADP) compared to the Control and Microwave Irradiation. The metabolizable protein (MP), truly digested protein in the small intestine (DVE), and feed milk value (FMV) were higher with Steam Pressure treatment. In Study 3, a higher absorbance in normal tannin genotypes for amide I and amide II peak height and area, structural CHO (STCHO), total CHO (TCHO) area and peaks (H_1015, H_1076, H_1145) and cellulosic compounds (CEC) to total CHO (TCHO) ratio. Amide I area and α-helix absorbance were not different among low tannin genotypes; however, higher ratios for α-helix to β-sheet height and CEC to STCHO were observed. Related to heat-induced changes, Steam Pressure treatment in low tannin genotypes increased ($P < 0.05$) the absorbance in amide I and amide II areas and α-helix peak height. Steam Pressure treatment also increased ($P < 0.05$) the absorbance in amide I and amide II area, the absorbance in the structural CHO (STCHO) area, cellulosic compounds (CEC) area and peak height (H_1235), CEC: TCHO ratio, and CEC: STCHO ratio and decreased the absorbance of all parameters related to total CHO (TCHO) spectral profiles. On the other hand, Microwave Irradiation increased ($P < 0.05$) the absorbance of all parameters related to total CHO spectral profiles, it reduced ($P < 0.05$) the absorbance in STCHO: TCHO ratio, and showed similar results to the Control for the rest of the spectral parameters. In general, based on the available data from this research, both low and normal tannin types grown in western Canada could be suitable protein and energy source ingredients in combination with other common feedstuffs in diet formulations for ruminants.

ACKNOWLEDGEMENTS

I would first like to thank my thesis supervisor Dr. Peiqiang Yu of the college of Agriculture and Bioresources at the University of Saskatchewan for the opportunity and valuable learning experience working in his team as a graduate student. Thank you greatly for your academic contribution, guidance, understanding, and trust required for the completion of this project. I want to expressly thank Zhiyuan Niu of the department of Animal Science for all the laboratory training, unconditional support, and continuous assistance during this research. I would also like to acknowledge the advisory committee members, Dr. Denise Beaulieu, Dr. Rex Newkirk, and research chair Dr. Tim Mutsvangwa for the useful suggestions, professional comments and friendly work environment which positively influenced the course of this work. I want to thank my work fellows Basim Refat, Walaa Gomaa, Yousef Khanfas and Luciana Prates for your instruction and help. To Lei Yaogeng, a special thanks for the software assistance and friendly help. I extend my gratitude to all staff, office, laboratory, and farm members of the college of Agriculture and Bioresources at the University of Saskatchewan. To my friend and life companion, Victor Guevara, thank you for the great support, patience, comprehension, teaching moments and life experiences during this demanding period. To my father Francisco Rodríguez, my mother Ma. Eugenia Espinosa, my brother Daniel Rodríguez, my niece and nephew Ma. Camila and José Antonio, my best appreciation for the unconditional love and encouragement through this process. You are the example, driving force and basis for this accomplishment, for that I dedicate this work to you. Finally, I would like to acknowledge the Government of Saskatchewan, Saskatchewan Pulse Growers, Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Feed Research Centre (CFRC), and the Rayner Dairy Research and Teaching Facility for the funding and resources to develop and complete this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

Abs	Absorbance
ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine (NRC Dairy model)
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BDNDF	Rumen bypass feed neutral detergent fiber
BST	Rumen bypass starch
CA4	Rapidly degradable carbohydrate fraction (sugar)
CB1	Intermediately degradable carbohydrate fraction (starch)
CB2	Intermediately degradable carbohydrate fraction (soluble fiber)
CB3	Available neutral detergent fiber or slowly degradable carbohydrate fraction (Digestible fiber)
CC	Unavailable neutral detergent fiber (Indigestible fiber)
CHO	Carbohydrates
CP	Crude protein
CLA	Cluster analysis
CT	Condensed tannin
D	Degradable fraction

Da	Dalton
dBDM	Intestinal digestibility of rumen bypass dry matter
dBNDF	Intestinal digestibility of rumen bypass fiber
dBST	Intestinal digestibility of rumen bypass starch
$DE_{p3\times}$	Digestible energy at a production level (3× maintenance)
dIDP	Intestinal digestibility of rumen bypass protein
DM	Dry matter
DPB	Degraded protein balance
DVBE	Truly absorbed bypass feed protein in the small intestine
DVE	Total truly digested protein in the small intestine (DVE/OEB system)
DVME	Truly absorbed rumen synthesized microbial protein in the small intestine
ECP	Rumen endogenous protein
ED_CHO	Effectively degraded carbohydrate
ED_N	Effectively degraded nitrogen
EDCP	Effective degraded crude protein
EDDM	Effective degraded dry matter
EDNDF	Effective degraded neutral detergent fiber
EDST	Effective degraded starch
EE	Ether extract (crude fat)
FMV	Feed milk value
IADP	Intestinal digestible rumen bypass protein
IDBDM	Intestinal digestible rumen bypass dry matter
IDBNDF	Intestinal digestible rumen bypass neutral detergent fiber

IDST	Intestinal digestible rumen bypass starch
Kd	Degradation rate of potentially degradable fraction
Kp	Passage rate
MCP_{RDP}	Microbial protein synthesized in the rumen based on rumen degraded protein
MCP_{TDN}	Microbial protein synthesized in the rumen based on available energy (total digestible nutrients at a production level)
ME	Metabolizable energy
$ME_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
MREE	Microbial protein synthesized in the rumen based on available energy
MREN	Microbial protein synthesized in the rumen based on rumen degraded feed crude protein
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for gain
$NE_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate
NPN	Non-protein nitrogen
OEB	Degraded protein balance (DVE/OEB system)
PA2	Rapidly degradable true protein (soluble true protein)
PB1	Moderately degradable true protein (insoluble true protein)
PB2	Slowly degradable true protein (fiber bound protein)

PC	Indigestible protein
RDNDF	Rumen degradable fiber
RDP	Rumen degradable protein
RUDM	Rumen undegradable dry matter
RUNDF	Rumen undegradable neutral detergent fiber
RUP	Rumen undegradable protein
S	Soluble fraction
SCP	Soluble crude protein
T0	Lag time
tdCP	Truly digestible crude protein
TDDM	Total digested dry matter
tdFA	Truly digestible fatty acid
$TDN_{1\times}$	Total digestible nutrients at a maintenance level
TDNDF	Total digested neutral detergent fiber
tdNDF	Truly digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrate
TDP	Total digested crude protein
TDST	Total digested starch
TMR	Total mixed ration
U	Rumen undegradable fraction
$uNDF_{288h}$	Undigestible neutral detergent fiber

1. GENERAL INTRODUCTION

In Canada, the use of grain legumes such as peas and faba bean has increased in the last decade as a suitable option for crop rotations as well as a good alternative protein and starch ingredient for animal diets. During the years, new genotypes of faba bean have been developed to obtain the best features to help both farm and crop producers to compete in the feed and food industries.

Getting faba bean into the established feed market is challenging as livestock animals are commonly fed with a mixture of grains and forages that are considered valuable among the industry to gain ideal animal performance. Regardless of the several benefits, the supply and demand of these feedstuffs are prone to change mainly because of diseases and variability in production and price. Generally, cereal grains in western Canada are susceptible to different mycotoxin diseases such as fusarium. These diseases limit the production of cereal grains and ban their use as edible ingredients in animal diets, causing economic losses in the crop and livestock industries. For the reasons previously mentioned, there is the need of alternative feed sources with high nutritive value and high nutrient availability to replace common feed ingredients, reduce cost of production, and increase animal productivity at the same time.

Several studies performed around the world and Canada have shown the effect of inclusion of faba bean mainly in poultry and swine diets. There is little or no scientific information of the effects in ruminants. Some studies performed in Canada included "Chemical composition of some faba bean cultivars" (Bhatty, 1974), "Value of whole plant faba bean silage as the sole forage for lactating cows" (McKnight and Macleod, 1977), "Ruminal Behavior of Protein and Starch Free Organic Matter of *Lupinus Albus* and *Vicia Faba* in Dairy Cows" (Yu et al. 2002b), "Characteristics and *in situ* degradability of whole crop faba bean, pea, and soybean silages" (Mustafa and Seguin,

2003), and “The potential role of annual forage legumes in Canada: A review” (McCartney and Fraser, 2010).

The nutritive value of the feeds can be determined by several methods, including chemical analysis to obtained nutrient profiles, *in situ* and *in vitro* procedures which are used for degradation and digestion studies. Additionally, modeling systems such as the National Research Council (NRC), the Cornell Net Protein and Carbohydrate System (CNCPS), and the Dutch model DVE/OEB provide helpful information and represent a complementary tool for livestock diet formulation.

Furthermore, several processing methods have been used to change the physical, molecular and nutritional characteristics of different feedstuffs with the purpose of making feed ingredients more digestible and available to livestock animals. The aim of heat treatment in ruminant feed is to modify their carbohydrate degradation and prevent nitrogen and energy losses in the rumen while improving microbial protein synthesis and increasing nutrient supply to the small intestine (Andrade-Montemayor et al. 2009; Yu et al. 2004).

A novel approach has been developed also to determine the chemical composition in feedstuffs. Infrared spectroscopy can be applied to reveal the molecular structure in a wide range of elements such as solids, liquids or gases. This modern technique is used as a common tool in the scientific fields of biology, physics, and chemistry as a relatively small amount of sample and little or no sample preparation is required. Moreover, this method of analysis is reagent-free and cost-effective. The molecular structure of feed nutrients is an important factor to understand the metabolic characteristics in feed analysis. The main purpose of FTIR technology is to determine the particular molecular structure inherent to each feed ingredient which is highly associated with feed quality and its nutrient utilization by the animal (Yu, 2005d).

Despite previous research there is the need for more detailed, updated and specific information about the actual nutrient profile and digestive behavior of newly developed faba bean genotypes grown in western Canada. Questions regarding true nutrient supply and metabolism of faba bean, the effect of replacing common feedstuffs such as cereal grains, peas, and canola meal with faba bean, the optimal levels of inclusion in ruminant diets, the potential effects of tannin level (low and normal), the impact of processing methods, as well as the association between molecular structure and nutrient utilization of faba bean still exist among the scientists and the livestock, feed, and crop industries.

The next chapter (Chapter II), includes a literature review of the global and local production of faba bean, their common uses in the industry as well as processing methods used to improve feed quality. This section describes the conventional feed evaluation methods, covering common nutrition prediction models such as CNCPS 6.5, NRC 2001 and DVE/OEB System. The basic principles and applications of Infrared spectroscopy in animal feed is also incorporated in this chapter.

Chapter III includes information and data analyses of the impact of genotypes and tannin levels (low and normal) on the physicochemical and nutritional characteristics of faba bean seeds grown in western Canada. In Chapter IV, information and results regarding the effect of heat processing methods on the physicochemical and nutritional characteristics of faba bean seeds grown in western Canada are described. Finally, Chapter V includes data of the molecular structure spectral analysis in faba bean, the heat-induced molecular structure changes, correlation and multi-regression evaluation as well as multivariate analysis of spectral results.

2. LITERATURE REVIEW

2.1. Global and local production of legumes and faba bean

Between 1998 and 2003 the largest producers of green faba bean seeds were Algeria, China and Morocco; the world production of green seeds for that period accounted to 940,000 tonnes/year while the production of dry seeds of faba bean was around 3.90 million tonnes/year. In 2005, approximately 2.6 million hectares were used for growth of faba bean in the world; 41 % of the total planted area was found in Asia, 33 % in Africa (mostly in Ethiopia), 12 % in Europe, 7 % in America, and 7 % in Oceania; China led the world production with 43 % of the 5.8 million tonnes produced in the same year (Douglas et al. 2013; Link et al. 2008). From 2006 to 2008 the total production of legume crops was 46.4 million tonnes with faba bean accounting for 3.3 million tonnes in this period (Akibode and Maredia, 2011; Singh et al. 2013). In the period from 2011 to 2013, 60 % of the total pulse world production was attributed to seven pulse producing countries, from which India led with 24.3 % of total production followed by Myanmar (7.3 %), Canada (7.0 %), China (6.3 %), Nigeria (4.6 %), Brazil (4.2 %), and Australia (4.2 %) (Joshi and Rao, 2017). In Canada, the production of pulses has been increasing since the 90's due to the growth of market opportunities, area expansion, and the implementation of new technologies by the producers (Singh et al. 2013). Presently, pulses are considered the fifth largest crop in the country, after wheat, barley, canola and corn. The main groups in charge and representation of pulse producers of Canada include the Alberta Pulse Growers Commission, the Saskatchewan Pulse Growers, the Manitoba Pulse Growers' Association, the Ontario Bean Producers' Marketing Board and the Ontario Coloured Bean Growers Associations (Government of Saskatchewan, 2018; Pulse Canada, 2017).

Prairie provinces in Canada have grown faba bean as soybean meal replacement for livestock diets since 1972 and Saskatchewan grew approximately 40,000 ha of faba bean in the years of 2014, 2015, 2016, and 2017 (Penner, 2018; Pulse Canada, 2017).

Ten years ago, the Crop Development Center (CDC) started a breeding program with faba bean for the potential markets of food and feed. Mainly, colored flower faba bean with larger seeds where exported to Sudan and Egypt to be used in the food industry; then white flower genotypes with smaller seeds were developed and introduced into the livestock system, mainly for swine diets. In 2015, the main genotypes grown in Saskatchewan were the low tannin genotypes (small seeds) CDC Snowdrop and Snowbird and the normal tannin genotypes FB9-4, Taboar, Florent and SSNS-1 (Fleury and Barker, 2015).

2.2. General background

2.2.1. History

Legumes are flowering plants of the Fabaceae or Leguminosae family which have been cultivated by humans for many decades. The seeds are easy to harvest and store which makes them a suitable crop to grow in different areas (FAO, 2016; Levetin and McMahon, 2008). The word "legume" comes from the Latin '*legumen*' that means seeds harvested in pods (Aykroyd and Doughty, 1982). Leguminous plants are also denoted as grain legumes or pulse crops. There are more than sixty species of grain legumes of which soybean, lupin, chickpea, lentil, cowpea and faba bean are among the most common kinds (Hedley, 2001). Pulses are the dried edible seeds of certain plants in the legume family. They are very high in protein and fiber, and low in fat. The United Nations Food and Agriculture Organization (FAO) recognizes 11 types of pulses grown worldwide. Crops that are harvested green such as fresh beans and peas are not considered pulses as the term "pulse" refers to edible dried seed of a legume. On the other hand, peanuts and soybeans are not pulses

because of their higher fat content while pulses contain nearly no fat (Pulse Canada, 2018; FAO, 2016). Pulses are warm season annuals that can tolerate most kinds of soils; they can grow in dry, humid or tropical lands, favoring the soil enrichment in diverse regions because of their ability to fix atmospheric nitrogen (FAO, 2016; Levetin and McMahon, 2008; Hedley, 2001). For many years, pulse crops have been used in the animal and food industries in several countries because of its significant source of nutrients such as vitamins, minerals, protein, starch and oil. Mature and immature seeds are commonly consumed by humans while the mature pods are usually used for flour and concentrate production (Schuster-Gajzágó, 2004).

Vicia faba, also known as broad bean, windsor bean, horse bean or faba bean has been cultivated for thousands of years around the world. Literature has demonstrated that this plant was first domesticated in the Near East and then spread to Central Europe, Russia, Eastern Mediterranean Coast, India and China; the presence in America was dated in the XVI century and later into Australia in the XX century (Cubero, 2011).

2.2.2. Plant and seed characteristics

Faba bean is a bushy and strong crop with square thick stems that can hold a plant of 1.5 meters tall. It has purple, white or pink flowers with clusters that can produce one (small seeded types) to six (large seed types) pods which are 15 to 20 cm long that usually have 3 to 6 seeds inside. Overall, small seeded genotypes grow approximately 60 pods per plant while this number decreases to 15 pods per plant in large seeded genotypes (Saskatchewan Pulse Growers, 2018; Etemadi et al. 2015).

Seeds of faba bean differ in shape and sizes among genotypes and pod location. Seeds can be classified as small, medium or large; usually, small seeds are referred as tic beans (*Vicia faba L.var. minor*), medium seeds known as horse beans or field beans (*Vicia faba var. equina*), and

larger seeds as broad beans (*Vicia faba var. major*). Broad beans are primarily cultivated for human food while horse beans are mainly used for animal feed in different countries (O'Kiely et al. 2017; Aykroyd and Doughty, 1982).

2.2.3. Growing conditions and characteristics

Faba bean is a long season or annual legume planted in winter or spring to be used like a whole crop or to obtain its edible beans. These crops grow better in humid and cool conditions but can tolerate frosty situations such as -10°C and up to -15°C; however, dry and hot climate can be damaging for the crop as faba bean cannot resist excessive heat during flowering. The ideal rainfall per year for growth is around 650 to 1000 mm and the adequate soil for production is medium textured with good moisture and a pH between 6.5 to 8.0 (Oplinger et al. 2017; Singh et al. 2013). *Vicia faba* represents a valuable crop to include in field rotations; the high nitrogen fixation capacity and the deep tap roots improves the soil's fertility and structure (FAR, 2012). Paddocks with higher nitrogen levels decrease nitrogen fixation activity as faba bean plants will mainly use nitrogen from the ground. Fixation of atmospheric nitrogen is important as it increases the residual nitrogen in the soil which will be used by future crops (Oplinger et al. 2017).

Faba bean in western Canada are usually seeded early in May to avoid the risk of heat or drought stress, to promote higher yields, and to obtain a higher percentage of mature pods (90%) by middle of September (Strydhorst and Olson, 2013).

The ideal temperature for seed germination is around 4°C (+-1), so planting should take place when the soil reaches this condition (Saskatchewan pulse growers, 2018a). Faba bean plant develops through different stages that include the seed emergence, the elongation time, the flowering period, the pod fill phase, the maturity process and the harvest process; usually flowering

happens between 45 to 60 days after emergence, and full maturity is reached around 110 to 130 days (Douglas et al. 2013; Saskatchewan pulse growers, 2018a).

2.2.4. Nutritional and genetic features

Pulse crops are a significant source of vitamins, minerals, protein, starch and oil. Seeds contain between 20%-25% of protein (mainly albumins and globulins), 39%-51% of starch (high amylopectin content), and 1%-2% of oil (mostly polyunsaturated fatty acids) (Schuster-Gajzágó, 2004).

Faba bean are rich in protein and starch. Protein values can vary depending on genetic resources or commercial genotypes. Starch is the main component and energy source (approximately 42%) and it shows a negative correlation with the protein level in the seeds. Faba bean have higher lysine content, lower fat and sugar content (1% and 4%, respectively), and lower cysteine, tryptophan, and methionine compared to cereal grains (Crepón et al. 2010). Low tannin genotypes can have higher crude protein and sugar levels, but lower starch, fiber, and fat compared to normal tannin genotypes. Crepón et al. (2010) pointed out that animal requirements can be met with a combination of faba bean and other sources of protein in the diet.

2.2.5. Condensed Tannins

Tannins are complex secondary plant metabolites widely distributed in plant species mainly as a resistant to pests and adverse environment. These phenolic compounds have a great number of phenolic groups (Jansman, 1993). Based on their chemical structure two main types of tannins are recognized, hydrolysable (HT) and condensed (CT). The former consists of polyphenols (gallic acid and ellagic acid) that can be easily hydrolyzed by heating with weak acid; on the other hand, condensed tannins are flavonoid polymers (catechins or proanthocyanidins) linked by carbon-carbon bonds that oxidize with hot mineral acid (Addisu, 2016; Hagerman et al. 1992).

Condensed tannins are polymerized products of flavan-3-ol (catechin) and flavan-3,4-diol (leucoanthocyanidins) or a mixture of both (Figure 2.1.). They can also be referred as flavolans or procyanidins (Jansman, 1993). Condensed tannins are capable of precipitating proteins and form soluble or insoluble complexes with various molecules (Addisu, 2016; Acuña et al. 2008; Frutos et al. 2004). These molecules are found in the cell walls or vacuoles in stems, leaves, flowers, or in the seeds of dicotyledonous plants. They vary greatly in their structure and composition which influence in the formation of different complexes. Some tannin factors such as their high molecular weight and structure flexibility promote the binding among molecules. The abundant number of phenolic groups in tannins is the reason for the high affinity with proteins as many bonding points with carbonyl groups are available. Relatively hydrophobic and large proteins with a flexible and open structure show more affinity for tannins. Protein-tannin complexes are generally unstable as the bonds continually break and re-form. The hydrogen bonds are stable at pH of 3.5 and about 8, but when values go above or below this point the bonds tend to breakdown (Bunglavan and Dutta, 2013).

a) b)

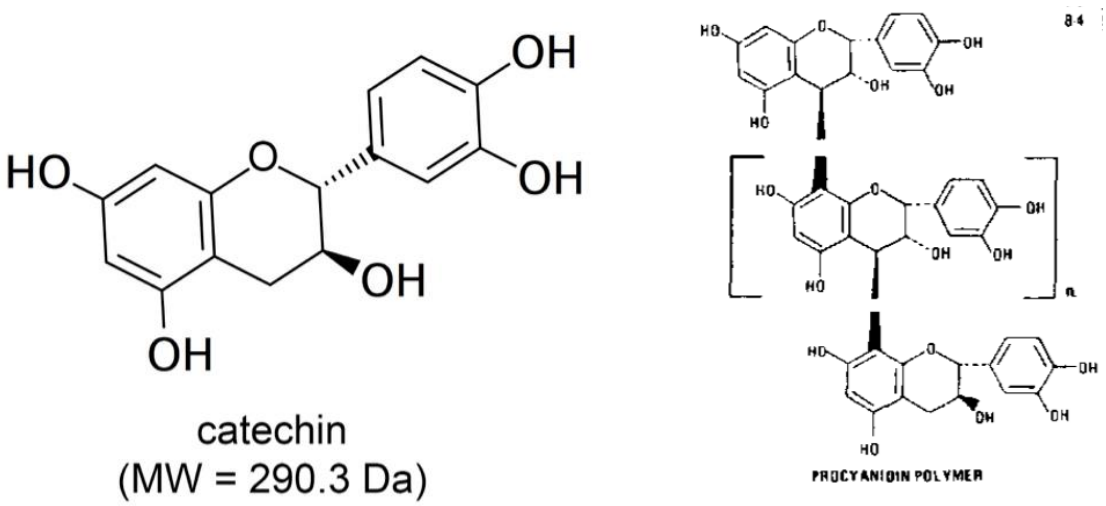


Figure 2.1. Tannin structure. a) catechin monomer, b) condensed tannin molecule. Source from Bianchi et al. 2016 and Jansman, 1993.

Many factors can influence the tannin concentration in legumes; relationships between tannin level and temperature of growth, plant species, soil fertility, plant pests and predators, the stage of plant maturity as well as the methodology used for analyses have been observed (Berard et al. 2011).

2.2.5.1. Effects of tannin content on animal performance

The response of animals to diets with tannin content has been studied and presents variable results of the detrimental and beneficial effects of its inclusion. The nutritional effects of tannins are mainly associated with their ability to bind with minerals, dietary and enzymatic proteins, and structural carbohydrates of cell walls in plants. The different tannin complexes formed with nutrients reduce their bioavailability in specific locations of the gastro intestinal tract (Adissu, 2016; Bunglavan and Dutta, 2013). Higher concentrations of condensed tannins (>80 g/kg DM) affect diet palatability; tannins can reduce feed intake as well as protein and dry matter digestibility as they are less available to the animal. Inclusions above 9 % can be lethal for most species (Berard et al. 2011). Hagerman et al. (1992) attributed the diverse digestion effects of tannins to differences in the physiology of animals to tolerate tannins and also to differences in the chemical reactivity of different types of tannins.

However, condensed tannins can also provide positive effects on protein degradation behaving as organic protectants of protein in animal diets. Some studies have reported that moderate levels of inclusion (1 % to 4 %) are beneficial for ruminants as they protect plant proteins from degradation by rumen microbes. Protected dietary protein in the rumen is linked with a lower production of ammonia nitrogen which increases protein availability in the small intestine. At moderate levels of inclusion negative impacts are not observed on dry matter intake and animal performance (Bunglavan and Dutta, 2013; Iqbal et al. 2011).

2.3. Main genotypes grown in western Canada

2.3.1. General background

Faba bean were first grown for commercial purposes in Canada at the end of 1960 in Nova Scotia. At the beginning of the 1970's, the first breeding program was performed in the Crop Development Centre in Saskatchewan. From the middle 1980 to now Limagrain corporation from the Netherlands had a main role in the development of the adapted genotypes grown in Canada (Kostuik, 2015).

Although, most cultivars of faba bean grown in Canada are produced by the Netherlands and Germany, the Crop Development Centre (CDC) is currently developing new breeding lines of both low and normal tannin faba bean with small and large seed sizes that can tolerate drier conditions and have more disease resistance (Heeg, 2017). In the present market, different genotypes of faba bean are grown for two main purposes. Normal or high tannin genotypes of the cultivars Taboar, CDC Fatima, Malik (FB 9-4), CDC SSNS-1, Florent, Fabelle, and Vertigo are generally gathered for human food and exportation; low tannin genotypes which commonly include Snowdrop, Snowbird, Imposa, and Tabasco are mainly used as feed for livestock (Saskatchewan Pulse Growers, 2018a; Phelps, 2015).

2.3.2. Variety/line description

a) **Snowbird:** This variety was obtained in 1989 in the Netherlands. For breeding selection main factors such as yield, early maturity, resistance to lodging and disease were considered. Snowbird gain national registration status in 2005 which is represented in Canada by Bob Park (CFIA, 2018a). This variety is 96 cm high and produces medium green foliage plants with white flowers; maturity is reached at 104 days when pods produce

small seeds (495 g/1000 seeds) with low tannin level. Dry seeds are elliptic and the color of the seed coat immediately after harvest is grey beige (CFIA, 2018b).

b) **CDC Snowdrop:** This faba bean variety was bred by Dr. Bert Vandenberg in the Crop Development Centre in the University of Saskatchewan in the city of Saskatoon and was released on 2012. Snowdrop is a low tannin plant of 98 cm in height which produces the smallest seeds among low tannin genotypes (335 g/1000 seeds). This cultivar is easier to seed, has high tolerance to lodging, and is adapted for all production areas. The plant reaches maturity at 104 days (Saskatchewan Pulse growers, 2018b; CFIA, 2018b).

c) **CDC 219-16:** This variety was developed in 2005 in the Crop Development Centre by Dr. Albert Vandenberg. The main characteristics for cultivar selection were based on days to flowering and maturity, plant yield and height, seed size and weight, seed coat color, and lodging. 219-16 is a mid-season flowering plant with a medium green foliage, white flowers and small seeds (360 g/1000 seeds). This variety is similar to Snowbird and CDC Snowdrop in terms of maturity but is shorter in height. The seeds are elliptic with low tannin content and immediately after harvest they have a grey beige color (Saskatchewan Pulse Growers, 2018c).

d) **CDC Fatima:** is a colored flower faba bean bred in the Crop Development Centre on 1994. The plant reaches maturity in 105 days. The stem is 106 cm tall and produces large seeds (520 g/1000 seeds) with normal tannin content (CFIA, 2018b; Fleury and Barker, 2015).

e) **Vertigo:** is a synthetic variety developed with four lines by DL Seeds Inc. in Morden, Manitoba. It has higher yield compared to CDC Fatima (PGDC, 2015). The plant takes 106 days to reach maturity. This faba bean is 107 cm tall, has colored flowers and large seeds (571 g/1000 seeds) with a normal tannin content (Saskatchewan Pulse Growers, 2018a).

f) **FB9-4:** This faba bean variety is known as Malik and was bred in the Crop Development Centre in Saskatchewan. The plants have colored flowers with large seeds (680 g/1000 seeds) which have a normal tannin content (Strydhorst and Olson, 2013). FB9-4 is 95 cm tall and reaches maturity in 104 days (Saskatchewan Pulse Growers, 2018a).

g) **346-10:** This line was bred by Dr. Albert Vandenberg in the Crop Development Centre (CFIA, 2018a). The plant has colored flowers with normal tannin level seeds.

h) **CDC SSNS-1:** This faba bean was registered on 2013 in the Crop Development Centre. SSNS-1 is a small seeded (335 g/1000 seeds), colored flower tannin variety; the plant is 109 cm tall and reaches maturity at 105 days (CFIA, 2018b; Fleury and Barker, 2015).

2.4. Faba bean used as animal feed

2.4.1. Main use in monogastric animals

In monogastric animals, the use of faba bean is not only decided by the level of tannin in the seed coats, but also by other components found within the seed such as vicine and convicine, which are considered antinutritional factors in this species. In case of adding faba bean to the diet formulation, lower levels of tannin and lower content of antinutritional components are desired. Several studies have shown the effects of different levels of faba bean inclusion mainly in poultry and swine diets. Nalle et al. (2010) completed research in broilers in New Zealand and concluded that faba bean can be added to balanced broiler diets without negative effects on performance or digestive tract development. Emiola and Gous (2011) in Nigeria determined no change in performance nor preference of dehulled faba bean meal vs. full fat soy diet in weaner pigs. In other studies, the use of faba bean had no negative impact on animal performance, meat quality or diet preference in pigs (White et al. 2015; Gatta et al. 2013). Processing of the seeds can help prevent

adverse effects of antinutritional factors in susceptible species and reduce the negative impact on digestibility of faba bean (Olson and Bowness, 2014; Douglas et al., 2013; Crepón et al. 2010).

2.4.2. Main use in cattle

Faba bean seem to be very palatable for cattle. The high starch content in the seeds make them a good energy source comparable to cereal grains, and as a protein source faba bean have been used as a suitable replacement for soybean meal in ruminant diets (Crepón et al. 2010). Inclusion in dairy cows had shown no negative impact on feed intake, milk yield or milk composition with 3.5 kg/animal per day (30 %) of ground faba bean (Douglas et al. 2013). In beef rations, studies showed no complications when using 90 % of faba bean as the main protein source (Crepón et al. 2010). In lambs, there was no change in daily gain or carcass quality when seeds where milled and mixed with lupin seeds (Lestingi et al. 2015). In buffalo nutrition, owners obtained high-quality meat with low fat, cholesterol, and saturated fatty acids at an inclusion level of 28 % of DM in the diet (Calabrò et al. 2014).

Although faba bean is a source of energy and protein, it can be easily and rapidly degraded by rumen microbes. This high degradability of protein and starch results in more nitrogen and energy loss in the rumen; as a consequence, less undegraded protein and starch is available for digestion and absorption in the small intestine. Fortunately, this digestive behavior can be controlled and improved by different processing methods that will reduce the degradability of this nutrients in the rumen, increasing their flow to the intestinal tract (Crepón et al. 2010)

2.5. Feed processing methods

2.5.1. General information

In the past, cattle were left in the field for grazing or they were fed cut forage and a moderate amount of grain for survival. Today, better animal performance and high production are the main

goal, and this requires higher levels of energy supplied generally by larger quantities of feed concentrates. Different feedstuffs used for livestock are primarily different in size, shape, texture, palatability, nutrient content and digestibility (Matsushima, 2006). This last factor is important as the majority of concentrate ingredients used for livestock have a large soluble fraction of starch and protein with a very high rate of degradation in the rumen; this effect, however can be changed with different processing methods which alter the physical, molecular and nutritional characteristics of feedstuffs (Goelema et al. 1999; Chae et al. 1997).

Processing of feed ingredients is usually divided into dry and wet methods. Grinding, pelleting, extruding, popping, micronizing, and roasting are classified within dry procedures while soaking, cooking, boiling, steam rolling, steam flaking, pressure cooking, and exploding are common wet methods for treating feeds (Matsushima, 2006).

2.5.2. Processing of legumes

Several processing treatments can reduce antinutritional factors such as lectins, condensed tannins, phenolic compounds, protease inhibitors, and alkaloids found in legumes; these treatments usually combine temperature, pressure, and moisture to alter the physical and molecular structure of the feedstuffs. In ruminants, the majority of feed processing methods will increase the surface area for bacterial attachment and enzymatic activity, making the feed more degradable in the rumen and increasing the nutritive value of legume seeds because of the increased glucose and protein supply into the small intestine (Matsushima, 2013; Andrade-Montemayor et al. 2009; Yu et al. 2004).

2.5.3. Heat treatments

In legumes, heat treatment can remove or inhibit antinutritional factors improving the quality of the seeds. However, heat can affect feed nutrients in diverse forms. The way of altering the protein degradative behaviour with heat involves mostly denaturation of the protein. Denaturation happens

when a denaturant is considerably large to breakdown the covalent and non-covalent bonds that keep together the protein structure. Heating results in protein structure stabilization and cross-linkages to carbohydrates. This effect protects proteins from ruminal hydrolysis, reducing their solubility and degradation rate in the rumen (Salazar-Villanea et al. 2016; Goelema et al. 1999). During the heating process, Maillard reactions can occur causing microbial resistance of proteins to proteolysis; however, these reactions are reversible in early stages when the temperature is moderate. In this case, rumen degradability of protein can be reduced without negative effects on digestibility (Andrade-Montemayor et al. 2009).

Thermal treatments affect starch structure in different ways. First, swelling of granules will occur as a result of the exposure to water and gradual heat increases (55°C). After cooling and drying the swelling is reversible, but when more temperature is applied (60 to 80°C), this process can become irreversible causing the loss of crystallinity in the starch granules by gelatinization. Partial or complete gelatinization may occur depending on temperature, moisture content, and different processing times. Starch degradability in the rumen can be increased when steam is used during the heating process. Other effects of thermal treatment on starch is the retrogradation, in which the molecules separated during gelatinization re-associate but without returning to its original form. Retrograded starch can result in the formation of a less digestible fraction than native starch. (Andrade-Montemayor et al. 2009; Goelema et al. 1999).

The main purpose of heat treatment of ruminant feeds is to modify their carbohydrate degradation and decrease the degradation of protein to prevent energy and nitrogen losses in the rumen while improving the production of microbial protein and increasing the nutrient supply to the small intestine (Andrade-Montemayor et al. 2009; Yu et al. 2004).

a) Steam Pressure Toasting

Pressure toasting uses pressurized steam to heat feedstuffs (Goelema et al. 1999). The effective heat transfer coefficient and the amount of condensation make the use of steam convenient during processing of feeds. Direct steaming at higher temperatures causes steam condensation on the surface of the external layer of seeds which can provide some kind of protection against overheating. "Toasting" usually refers to the use of steam to achieve temperatures around a 100°C. This is a cost-effective method, frequently used in the feed industry for processing legumes and some oilseeds, as it is effective for oil extraction and the reduction of some antinutritional factors. To determine the effects of high temperatures over nutrients, autoclaving has usually been used (Van der Poel et al. 1990). Autoclaving can change the chemical profiles, metabolic characteristics, and molecular structure of the feeds by the combination of moisture, heat, and pressure (Ying, 2015).

b) Microwave Irradiation

Microwave Irradiation is an effective method used for processing and drying food and feed. Heating in a microwave is unique as heat is caused by molecular friction of electrical dipoles in a fluctuating electric field of specific frequency (Fakhouri and Ramaswamy, 1993). The energy produced by a microwave is non-ionizing and interacts with polar molecules and charged particles of the medium, changing its electromagnetic field and increasing the temperature rapidly. Microwaves have high penetration power and energy efficiency as most electro-magnetic energy can be converted into heat. These advantages shorten processing times and accelerate heating rates compared to conventional heating where heat is normally transferred from the surface to the interior (Maheri-Sis et al. 2011; Sadeghi and Shawrang, 2005).

2.6. Conventional feed evaluation methods

2.6.1. Determination of condensed tannins

Techniques for measuring tannin content of plants were first developed for the leather industry. The increased interest in these bio-active compounds led to the development of more sophisticated and precise analytical methods of identification (Tempel, 1982). Tannin quantification techniques are usually based on the chemical properties and the ability of tannins to bind to substrates (FAO/IAEA, 2000). Different procedures are available to determine tannin concentration in feeds, chemical methods are usually used for the determination of condensed tannins, total phenolics, total tannins and gallotannins; other methods of identification are based on tannin protein precipitation or binding characteristics (FAO/IAEA, 2000). The HCl butanol method is a simple and commonly used procedure to quantify condensed tannins; the basis of the essay is the colorimetric reaction produced by the released of anthocyanidin when the flavonoid links are broken in an acidic environment. The isolation of purified condensed tannins, specific to plant species and tissues are necessary for a complete identification of this molecule as the differences in the structure of condensed tannins can produce different reactivities in the analysis (Shay et al. 2017). Adjustments to this procedure have been done to avoid negative results associated with the interference of chlorophyll and other pigments; iron inclusion in the essay can increase the reproducibility and sensitivity of the method (FAO, 2000; Hagerman, 2002).

2.6.2. Cornell Net Carbohydrate and Protein System

The Cornell Net Carbohydrate and Protein System (CNCPS) is a mathematical model developed at by the University of Cornell, University of Pennsylvania and Miner Institute (CPM dairy) to estimate cattle nutrient requirements and supply, signifying a useful tool for diet evaluation and formulation (Fox et al. 2004). The first version was released in 1991 and was developed based on

animal physiology, rumen function, microbial growth, feed digestion and passage in different conditions of production. To predict the degree of rumen degradation, microbial protein synthesis, intestinal absorption, and total metabolizable energy and protein supply, CNCPS uses data of feed protein and carbohydrate degradation. Cattle requirements predicted by the model account for management, environmental, and feed characteristics, as well as the animal body reserves and different physiological periods such as growth, pregnancy, and lactation (Van Amburgh et al. 2013; Van Amburgh et al. 2010).

Since the first version, several updates have been performed to improve research and diet formulation. The continuous model development has contributed to the prediction of performance with great precision. Important updates included refining of the feed library and equation improvement to predict nitrogen excretion (Van Amburgh et al. 2010; Van Amburgh et al. 2013). CNCPS 6.5. version has been available since 2015. The actual software includes a feed library with values for protein, carbohydrates, fiber, amino acids, volatile fatty acids, vitamins, and minerals. Also, data of rates of degradation and intestinal digestibility of protein and carbohydrate fractions of some feeds can be found (Ying, 2015). Knowledge of on farm specific characteristics, environmental factors, and feeds used in different production conditions contributes to a more accurate prediction of nutrient requirements for growth, maintenance, production and nutrient excretion in cattle (Fox et al. 2004).

CNCPS system has been used as a field management instrument to optimize on farm feed growth and herd size, increasing the economic return. In several evaluations, CNCPS had shown to be advantageous to predict animal responses to a certain diet as well as animal performance and production (Van Amburgh et al. 2015; Higgs et al. 2015; Tylutki et al. 2008; Lanzas et al. 2007; Fox et al. 2004).

2.6.3. Energy value estimation in feed ingredients

Energy values and availability from different feedstuffs are necessary to determine the amount of feed needed by a lactating cow. Energy requirements for different activity levels, physiological states, and environmental stress must be considered as well to establish the amount of energy required for body maintenance or production in cattle (Tyrrell, 2005; Eastridge, 2002).

The prediction of energy values in ruminants have been achieved by including both animal and feed components into the dynamic NRC dairy model (Ying, 2015; Eastridge, 2002).

In the last edition of the Nutrient Requirements of Dairy Cattle - NRC 2001, feed energy values are obtained and expressed by using the total digestible nutrient (TDN) system based on actual feed composition data (NRC, 2001). TDN is obtained by adding together values of digestible protein, digestible carbohydrates (NDF, non-structural carbohydrates), and digestible fat, all of them on dry matter basis (Tyrrell, 2005).

High milking cows consume at least three or four times their maintenance intake. Feed digestibility in dairy cows is reduced when feed intake is increased, which decreases the energy value of the diet. For this reason, the NRC model considers a percentage discount for digestibility when calculating the digestible energy at productive levels of intake (DE_p). To determine net energy values of lactation (NE_L), NRC uses data of the actual intake (3x and 4x maintenance) and whole diet digestibility; metabolizable energy (ME_p) at production levels of intake is determined based on DE_p. The National Research Council (NRC, 1996) is used to obtained results for net energy of maintenance (NE_m) and net energy of gain (NE_g) (NRC, 2001; Ying, 2015).

2.6.4. In situ technique for estimation of rumen degradation kinetics of feed nutrients

The artificial nylon bag or rumen bag technique is a valuable method for a primary evaluation of feed ingredients. This method has been used by researchers to study several characteristics of degradation processes within the rumen and to determine the degradability of different feeds (Ørskov et al. 1980). The *in situ* technique has been proven as a convenient and simple procedure to detect the extent and rate of degradation of primary nutrients such as protein and starch in the rumen. This information is important to understand better the degradation in the rumen (Ørskov et al. 1980; Ying, 2015).

2.6.5. Three step in vitro technique for evaluation of intestinal digestibility in feed nutrients

Total protein available for absorption in the small intestine depends on the flow of microbial protein and dietary nitrogen, as well as their intestinal digestibility. The digestibility of bypass protein and amino acid profile varies among feed ingredients (Wang et al. 2015; Calsamiglia and Stern, 1995).

The *in vitro* protocol was recommended by Calsamiglia and Stern (1995) because of the need for more precise protein digestion estimations in feeding systems; later this essay was modified by Gargallo et al. in 2006. When the *in vitro* method was compared to *in vivo* procedures, it proved to be reliable, less expensive, making it suitable as a routine essay for digestibility evaluations (Wang et al. 2015). The importance of this technique is that it can reproduce the physiological conditions of the animals, showing an estimate of the absorbed dietary protein from specific feed ingredients in the small intestine allowing it to be used for feed quality control and determination of general values of protein supplements in ruminant diets (Calsamiglia and Stern, 1995).

2.6.6. Prediction of truly digestible protein supply to the small intestine in dairy cattle

2.6.6.1. DVE/OEB System

The DVE/OEB system is a protein evaluation model that was introduced in the Netherlands in 1991 and later published by Tamminga et al. (1994). Based on the digestion and metabolism of N and the digestive behaviour of several feeds, this system provides useful information for providing protein to accommodate real demands of the dairy cattle and prevent unnecessary nitrogen losses (Tamminga et al. 1994).

In DVE/OEB, the requirements for dairy cows and the protein value of feedstuffs are determined by the amount of feed and microbial protein that can be truly digested and absorbed in the small intestine of cattle (Yu et al. 2003). For each feed, the model includes two main values: first the DVE or truly digested protein in the small intestine (g/kg) that is obtained from the truly digested microbial protein in the small intestine (DVME) plus the truly absorbed rumen bypass protein in small intestine (DVBE) minus a correction for endogenous protein loss in the digestive tract (ENDP), associated to the amount of undigested dry matter observed in the feces; DVE = DVME + DVBE – ENDP. The second value is the OEB or degraded protein balance which represents the difference between the potential microbial protein synthesis originated from the degraded protein in the rumen (MREN) and the one that accounts for the available energy for microbial fermentation in the rumen (MREE) where, MREN = CP × [1 − (1.11 × RUP (% CP)/100)]; 1.11 is a factor that represents the regression coefficient of *in vivo*, on *in situ* degradation data; MREE = fermented organic matter (FOM g/kg) × 0.15 (Tamminga et al. 1994). When the OEB value is negative, it represents a lack of rumen N, while if positive indicates potential N loss from the rumen. DVE and OEB values are mainly used to meet protein requirements in different physiological stages when formulating dairy cattle feed (Yu et al. 2003).

2.6.6.2. NRC Dairy 2001 Model

Seven editions of Nutrient Requirements for Dairy Cattle have been published since 1944 with the last update version presented in 2001 in which new technology and information is applied related to modern concern in the dairy cattle field (NRC, 2001). In this model, the concepts of metabolizable protein (MP) and rumen degraded protein balance (DPB) are introduced to predict the truly digested and absorbed protein in the small intestine of dairy cows. MP consists mainly of the truly absorbed rumen undegraded protein in the small intestine (ARUP), the truly absorbed microbial protein in the small intestine (AMCP), and the truly absorbed rumen endogenous crude protein (AECP) from saliva, respiratory tract, and the digestive tract; MP = ARUP + AMCP + AECP (Ban, 2016; Ying, 2015; Yu et al. 2003; NRC, 2001). The DPB value is calculated accounting for the potential microbial protein synthesis based on the ruminally degraded feed protein and the microbial protein synthesized in the rumen based on available energy TDN: DPB (g/kg of DM) = $RDP_{NRC} - 1.18\ (MCP_{TDN})$.

2.6.7. Feed milk value in dairy cattle

The ability of cows to convert feed nutrients into milk or milk components is known as feed efficiency. It represents the kilograms of milk produced per kilogram of dry matter consumed (Heinrichs et al. 2018). Based on metabolic characteristics from the NRC and DVE/OEB protein systems, the feed milk value (FMV) can be determined. Values of total metabolizable protein (MP) of the feed, as well as fixed values of assumed efficiency of use of MP for lactation (0.67) and milk protein content (33 g protein / 1 kg of milk) are used for the calculations of FMV (Guevara, 2017).

2.7. Mid-Infrared spectroscopy techniques in feed science

2.7.1. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared (IR) spectroscopy has been available since the 1940's and has been developed into an important analytical technique for modern researchers. It is a non-destructive and fast procedure for the analysis of feed chemical composition. One advantage of IR spectroscopy is that practically any sample, in any form can be analyzed. Improvements in the time of analysis and the quality of the infrared spectra were made with the introduction of Fourier transform spectrometers (Sun et al. 2018a; Stuart, 2004). The basic elements of a spectrometer include a source of radiation, an interferometer, a detector, an amplifier, a converter (analog to digital), and a computer. The interferometer will produce a signal related to the interference of radiation or change of pathlength between two beams. The distance and frequency are interconvertible by the mathematical method of Fourier-transformation (Stuart, 2004).

The interferometer used in FTIR (Figure 2.2.) involves two perpendicularly plane mirrors, a beam-splitter which is a semi-reflecting film that divides the surface of the mirrors. When radiation goes through an ideal beam-splitter, 50% of radiation will be reflected in each of the mirrors. The two beams are reflected from these mirrors, returning to the beam-splitter where they recombine and interfere (Stuart, 2004). The transmitted beam is the one detected in FTIR spectrometry and it is the gleam that appears from the interferometer at 90° to the input beam (Stuart, 2004).

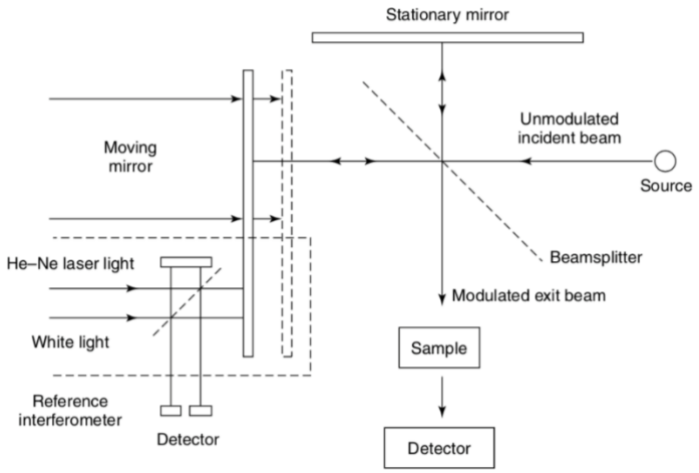


Figure 2.2. Fourier Transform Infrared Spectrometer (FTIR) interferometer. Source from Stuart, 2004.

2.7.2. Basic principles

The basis of this technique is related to the atom vibrations within a molecule. In IR spectroscopy, the identification of different chemical functional groups and spectra are obtained by passing infrared radiation through a sample to determine different molecule energy absorptions (Sun et al. 2018a; Stuart, 2004). Yang and Yu (2016), indicate that the infrared radiation absorption is proportional to the changes in energy due to different kinds of vibration in the molecules like bending or stretching. To detect inherent molecular structure of a specific sample, attenuated total reflectance Fourier transform infrared (ATR-FTIR) micro spectroscopy is usually used (Sun et al. 2018a). The IR region includes three main areas: near-IR (ca. 13,000 to 4,000 cm^{-1}), mid-IR (ca. 4,000 to 200 cm^{-1}), and far-IR (200 to 10 cm^{-1}); generally, the mid-IR region is the mostly used for analysing samples (Yang and Yu, 2016).

2.7.3. Application of FTIR technique in feed analysis

Infrared spectroscopy can be applied to reveal the molecular structure in a wide range of elements such as solids, liquids or gases. This modern technique is used as a common tool in the scientific fields of biology, physics, and chemistry as a relatively small amount of sample and little or no sample preparation is required. Moreover, this method of analysis is reagent-free and cost-

effective. The molecular structure of feed nutrients is an important factor to understand the metabolic characteristics in feed analysis. FTIR-ATR can be used to study the biomolecular spectral features of feeds due to gene alteration or heat processing changes without the damage related to the reagents used in common chemical analysis. The molecular characteristics of feeds and their inherent structure are highly related with feed quality, nutritive value, and nutrient utilization (Yang and Yu, 2016; Yu, 2005c; Stuart, 2004).

2.7.4. Spectral analysis methods

The primary statistical methods used to understand the spectral data of feed samples include univariate and multivariate analyses. The univariate analysis is the most direct method for spectral data interpretation based on the evaluation of the frequency and intensity (peak heights and areas) of specific functional groups such as amide I, amide II, aromatic compounds, and cellulosic compounds; this data can be related with the chemical components of a feed. Nevertheless, the use of univariate analysis does not always determine accurately the location and concentration of the functional groups (Ban, 2016; Yu, 2005a).

In the multivariate approach, multiple variables can be analyzed at the same time to classify and differentiate the sample's molecular structure and molecular structure changes. The common procedures for multivariate analysis include cluster analysis (CLA) and principal component analysis (PCA) (Yu, 2005a).

Cluster Analysis (CLA) method groups spectra results based on similarity with other spectra and shows data as dendrograms based on a distance matrix. Groups of data are obtained by pairing the most similar objects into a new cluster or hierarchical group; new forming groups will be integrated into the previous one until one general group is created (Ban, 2016; Ying, 2015; Yu, 2005a).

Principal Component Analysis (PCA) is a statistical data reduction method mainly used to determine major sources of variation in the FTIR spectra. In this analysis the original data set is transformed to a new set of uncorrelated variables known as "principal components" (PCs). The aim of this method is to obtain one or two independent linear combinations (PCs) while keeping as much information from the original variables; the first few PCs mostly account for 95% of the variance. The results from PCA are presented in two-dimensional (2 PCs) or three-dimensional (3 PCs) scatter plots (Yu, 2010).

2.8. Literature review summary, research objectives and hypotheses

2.8.1. Summary

Legumes are flowering plants of the Fabaceae or Leguminosae family which have been cultivated by humans for many decades. Pulses are warm season annuals that can tolerate most kinds of soils as they can grow in dry, humid or tropical lands, enriching the soil in diverse regions.

Faba bean is rich in protein and starch. Starch is the main component and energy source in the seeds (approximately 42%) and it shows a negative correlation with protein content. Faba bean is high in lysine content, low fat and sugar content, and low cysteine, tryptophan, and methionine compared to cereal grains (Crepón et al. 2010). Low tannin seeds have higher crude protein and sugar levels, lower starch, fiber, and fat than high tannin seeds. Crepón et al. (2010) pointed out that animal requirements can be met with a combination of faba bean and other sources of protein in the diet.

The easily and rapid degradability of protein and starch of faba bean in the rumen provides less amino acids and glucose to be digested and absorbed in the small intestine. Fortunately, the digestive behavior of feeds can be controlled and improved by different processing methods that

can reduce the degradability of nutrients in the rumen, increasing their flow to the intestinal tract (Crepón et al. 2010; Yu et al. 2000).

Complementary methods for feed analysis such as the application of FTIR techniques can help in the understanding of the molecular characteristics related to the specific inherent structure of feed components, or the molecular structure changes produced by processing methods with the main goal of feed quality control, determination of the nutritive value of different feeds, and enhancement of nutrient utilization in dairy cattle.

2.8.2. Research objectives

General

Determine the effect of genotypes, heat processing methods, and tannin levels (low and normal) on the structural, physicochemical, and nutritional characteristics of faba bean grown in western Canada.

Long-term

1. To efficiently utilize different genotypes of faba bean as an alternative feed in cattle production systems for improving animal production and performance in western Canada.
2. To increase basic knowledge of the nutritional characteristics and relevance of faba bean seeds to apply this information into high quality feeding programs and to aid faba forage and faba bean breeding programs.
3. To develop feeding strategies to maintain and increase business in a competitive market for pulse producers and industry, so they can maximize their profit.

Short-term

1. To systematically study chemical, energy values, nutrient fractions, ruminal degradation kinetics, intestinal digestibility, nutrient availability, and true nutrient supply of newly

developed genotypes of faba bean with low and normal tannin levels grown in western Canada.

2. To obtain all necessary feed nutritional parameters for various advanced nutrient and diet formulation models: CNCPS, NRC, and/or DVE.
3. To reveal molecular structure features and heat processing induced molecular structure changes that affect nutrient utilization and availability in cattle using molecular spectroscopic techniques.

2.8.3. Research hypothesis

1. Genotypes of faba bean seeds, feed processing treatments and tannin levels (low and normal) dramatically impact the structural, physicochemical, and nutritional characteristics of faba bean grown in western Canada.
2. Heat processing methods will significantly modify the metabolic structure of faba bean seeds, altering the nutritional values, sites of digestion and nutrient utilization in dairy cattle.
3. Different genotypes grown in western Canada show different responses to different heat processing methods, affecting the ruminal degradation and intestinal digestion behaviors in dairy cattle.
4. Molecular structural changes related to heat processing can be revealed with FTIR molecular spectroscopy technique and are highly associated with nutrient availability of faba bean in dairy cattle.

3. IMPACT OF GENOTYPES AND TANNIN LEVELS (LOW AND NORMAL) ON THE PHYSICOCHEMICAL AND NUTRITIONAL CHARACTERISTICS OF FABA BEAN SEEDS GROWN IN WESTERN CANADA.

3.1. Abstract

The aim of this study was to evaluate the impact of genotypes and tannin levels on the physicochemical and nutritional characteristics of faba bean as an alternative protein and energy source for ruminants. Eight genotypes with two tannin levels (low vs. normal) grown in three different locations in Saskatchewan were analyzed. Chemical analyses were performed following the AOAC standard methods, energy parameters were evaluated using NRC-2001. Rumen degradation kinetics were determined using the standard *in situ* animal trial procedure according to the 'gradual addition/all out' schedule for 0, 2, 4, 8, 12 and 24 hours. Intestinal digestion was analyzed using the three-step *in vitro* technique with 12 hours pre-rumen incubation sample. The experimental design was RCBD (genotypes as a fixed effect and locations as a random block effect). Procedure of MIXED model of SAS 9.4 was used for statistical analyses with significance declared at $P < 0.05$ and tendency when $P < 0.10$. SAS contrast was used to compare low tannin and normal tannin genotypes. Results showed there were no differences ($P > 0.10$) in dry matter (DM), protein (CP), or carbohydrates (CHO) content between normal vs. low tannin types with an average of 92.8, 28.3, and 67.7 % DM, respectively. Low tannin faba bean (LT) had higher ($P < 0.05$) content of soluble crude protein (SCP) and non-structural carbohydrates (NSC); they also showed highly significant difference ($P < 0.05$) in the rapidly degradable protein fraction (PA2), rumen bypass starch (BSt), intestinal digestibility of rumen bypass protein (dIDP) compared to NT. On the other hand, normal tannin faba bean (NT) had more organic matter (OM), acid detergent lignin (ADL), higher slowly degradable protein fraction (PB2), and total tract digested

starch (TDST) compared to LT. No significant differences ($P > 0.10$) were observed for total digestible nutrients (TDN_{1x}), metabolizable protein (MP), feed milk value (FMV), or rumen undegraded crude protein (RUP). Even when results showed differences in physicochemical characteristics among faba bean seeds, the predicted animal performance was not different. These outcomes suggest that faba bean can be used as nutritive ingredient for dairy cattle without a significant variety or tannin level effect on metabolic characteristics.

3.2. Introduction

In Canada, the use of grain legumes such as peas and faba bean have increased in the last decade as a suitable option for crop rotations as well as a good alternative protein and starch ingredient for animal diets. the Crop Development Center (CDC) has been a great contributor to the market; new genotypes of faba bean have been developed to obtain the best features to help both farm and crop producers to compete in the feed and food industries.

Vicia faba, commonly known as faba bean is an annual crop planted in winter or spring to be used like a whole crop or to obtain its edible beans. These crops grow better in humid and cool conditions but can tolerate frosty situations such as -10°C and up to -15°C. (Singh et al. 2013; Oplinger et al. 2017). Faba bean is a valuable crop, as they improve the fertility and structure of the soil because of their high nitrogen fixation capacity and their deep tap roots (FAR, 2012).

Faba bean is rich in protein and starch. Protein values can vary depending on genetic resources or commercial genotypes. Starch is the main seed component (approximately 42%) and it shows a negative correlation with the protein level in the seeds. Faba bean has a higher lysine content, lower fat and sugar content (1% and 4%, respectively), and lower cysteine, tryptophan, and methionine compared to cereal grains (Crepón et al. 2010). Low tannin genotypes can have higher crude protein and sugar levels and lower starch, fiber, and fat compared to normal tannin

genotypes. In general, faba bean has a good nutritional profile compared to other legumes, and so they can compete with other protein feeds such as soy bean meal, lentil, peas and canola meal (Douglas et al. 2013). Crepón et al. (2010) stated that animal requirements can be met with a combination of faba bean and other protein sources. However, faba bean protein is easily and rapidly degraded by rumen microbes resulting in more potential nitrogen and energy loss in the rumen, producing less undegraded protein and starch available for digestion and absorption in the small intestine. Additionally, some faba bean genotypes contain condensed tannins which might interfere with the absorption of feed nutrients, and so limit the use of faba bean as regular animal feed, especially in monogastrics (Adamidou et al. 2011).

To our knowledge, there is no detailed information on chemical profiles, nutrient utilization and availability in dairy cattle in recently developed faba bean genotypes grown in western Canada. For this reason, the present study aimed to evaluate the effects of genotypes and tannin levels (low and normal) on the degradation, intestinal digestion and availability of nutrients in dairy cattle.

3.3. Study objectives

1. To determine the detailed chemical profiles of new genotypes of faba bean grown in western Canada to obtain a scientific base for future studies.
2. To determine the nutritional and metabolic characteristics of low and normal tannin faba bean and the impact on nutrient degradability and availability in dairy cattle.

3.4. Study hypotheses

1. New lines and genotypes of faba bean grown in western Canada would have significantly different nutritional characteristics, providing a different nutrient supply to dairy cattle.
2. Tannin content in low and normal tannin faba bean would significantly affect the nutrient rumen degradability and availability in the small intestine of dairy cattle.

3.5. Materials and Methods

3.5.1. Ingredients and sample preparation

A total of 24 samples were used in the present study. 8 genotypes (3 low tannin + 5 normal tannin) grown in 2016 at 3 different locations in Saskatchewan (Meath Park, Outlook and Rosthern) were analyzed. Low tannin faba bean included: Snowbird, CDC Snowdrop, and CDC 219-16; for the normal tannin faba bean the following genotypes were used: CDC Fatima, Vertigo, FB9-4, 346-10, and CDC SSNS-1.

All samples were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) before performing the chemical analyses.

3.5.2. Chemical analyses

3.5.2.1. Condensed Tannins (CT)

The HCl butanol method based on Porter et al. (1986) was used to determine CT levels in the present study. This assay is based on a colorimetric reaction to produce an oxidative depolymerization of condensed tannins by using an acid to obtain red anthocyanidins (Schofield et al. 2001). This procedure is the most common for determining CT in plant material, still some considerations and limitations of this assay are described in Schofield et al. 2001. A summary of the procedure is presented in the next paragraph.

The ground samples were weighed into test tubes (4 tubes/ sample) and then HCL butanol reagent was added to each tube, covered with marbles and vortexed. 3 of 4 tubes were incubated for 60 min at 97° C. in a water bath (Precision, Serial Nº: 602071249, Thermo Scientific, USA) and vortexed every 10 min. After incubation, all tubes were centrifuged for for 10 min at 3000 rpm and then read with a Spectrophotometer (Spectra max- 384 plus, Molecular devices USA) at

a wavelength of 550 nm (Porter et al 1986; Theodoridou, 2010). A complete description of the procedure is included in the appendix section.

3.5.2.2. Nutrient profiles

Nutrient profiles were obtained using the following standard methods:

- Dry matter (DM) - AOAC 930.15 (AOAC, 2005).
- Ash - AOAC official method 942.05 (AOAC, 2005).
- Protein: CP - AOAC official method 984.13 (AOAC, 2005); neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble protein (ADICP) were determined using NDF (without sodium sulfide) and ADF residues, following the procedure by Licitra et al. (1996); NPN (Licitra et al. 1996); soluble crude protein (SCP) was determined by incubating the samples at 39° C with borate-phosphate buffer and filtering through a Whatman #54 filter paper as described by Roe et al. 1990.
- Soluble carbohydrates: sugars - AOAC official method 974.06 (AOAC, 1990); starch was determined following the developed Megazyme total starch assay using the α amylase/amyloglucosidase method (AOAC 996.11, AACC 76.13, ICC standard method No. 168).
- Fiber and lignin: Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were analyzed using ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY), according to Van Soest et al. (1991); cellulose (ADF - ADL) and hemicellulose (NDF - ADF) were estimated according to NRC (2001).
- Lipids: EE - AOAC official method 920.39 (AOAC, 1990).
- Total carbohydrates (CHO) were calculated as 100 – (ash + CP + EE) (NRC, 2001).

- All samples were analyzed in duplicates. Analysis were repeated when results showed more than 5 % of error.

3.5.3. Determination of protein and carbohydrate sub-fractions (CNCPS 6.5)

In the Cornell Net Carbohydrate and Protein System referred to in Higgs et al. (2015) and Van Amburgh et al. (2015), protein is partitioned into ammonia and soluble true protein (PA), moderately and slowly degradable true protein (PB), and indigestible protein (PC), all based on their rumen degradation characteristics.

CNCPS v6.5 protein fractions are described as follows:

- PA1 fraction (ammonia) calculated as: PA1 = ammonia × (SP/100) × (CP/100). Degradation rate (Kd) of 200 %/h.
- PA2 fraction (soluble true protein) calculated as: PA2 = SP × CP/100 − PA1. Kd: 27 %/h.
- PB1 fraction (insoluble true protein) calculated as: PB1 = CP − (PA1 − PA2 − PB2 − PC). Kd: 12 %/h.
- PB2 fraction (fiber-bound protein) calculated as: PB2 = (NDICP − ADICP) × CP / 100. Kd: 5 %/h.
- PC fraction (indigestible protein) calculated as: PC = ADICP × CP / 100.
- Carbohydrates are partitioned into different fractions based on rumen degradation and microbial activity on available carbohydrates (Lanzas et al. 2007; Van Amburgh et al. 2010) into volatile fatty acids (CA1), lactic acid (CA2), other organic acids (CA3), rapidly degradable carbohydrates (CA4), intermediately degradable carbohydrates (CB1 and CB2), available neutral detergent fiber or slowly degradable carbohydrate fraction (CB3), and unavailable neutral detergent fiber (CC).
- CNCPS v6.5 CHO fractions are described as follows:

- CA1 fraction (volatile fatty acids) determined as: CA1 = Acetic + Propionic + Butyric + Isobutyric. Not degradable (0 %/h).
- CA2 fraction (lactic acid). Kd: 7 %/h.
- CA3 fraction (other organic acids). Kd: 5 %/h.
- CA4 fraction (soluble sugars). Kd: 40 %/h.
- CB1 fraction (starch). Kd: 25 %/h.
- CB2 fraction (soluble fiber) calculated as: $CB2 = NFC - CA1 - CA2 - CA3 - CA4 - CB1$. Kd: 30 %/h.
- CB3 fraction (digestible fiber) calculated as: CB3 = aNDFom – CC. Kd: 5 %/h.
- CC fraction (indigestible fiber) calculated as: CC = uNDF (288 h).

Specific degradation values of protein and carbohydrate fractions for this project were obtained from the CNCPS feed library from NDS software v6.5.

3.5.4. Determination of energy values

Using previous results obtained from chemical profiles, NRC dairy (2001) was used to determine the energy values of the samples. Analyses were based on TDN fractions, including: truly digestible CP (tdCP), truly digestible non-fiber carbohydrates (tdNFC), truly digestible NDF (tdNDF), and truly digestible fatty acids (tdFA), total digestible nutrients at 1x maintenance (TDN_{1x}) digestible energy at a maintenance level (DE_{1x}), digestible energy of production at 3x maintenance level (DE_{p3x}), metabolizable energy of production at 3x maintenance level (ME_{p3x}), and net energy of production at 3x maintenance level (NEL_{p3x}). To determine metabolizable energy (ME), net energy for maintenance (NE_m), and net energy for gain (NE_g), NRC beef (1996) was used.

3.5.5. Rumen in situ incubation procedure and rumen degradation kinetics of feed nutrients

Animals and sample preparation:

Four cannulated Holstein Friesian milking cows were used for the *in situ* study. The cows were kept in tie stalls during the period of sampling; they were milked three times a day in a milking parlor and fed two times daily with a total mixed ration (TMR) based on barley silage, hay, energizer, pellets and rolled barley; the average daily intake was 28 kg of DM. Detailed information on the cows is presented in Table 3.1. This trial was performed in the Rayner Dairy Research and Teaching Facility in Saskatoon, SK, Canada.

Samples were ground in the Canadian Feed Research Centre (CFRC) in North Battleford, SK, Canada, using the telemecanique roller mill (Emerson, Poland) with a roller gap of 0.508 mm. 7.5 g of sample were weighed into 10 x 20 cm nylon bags with a size pore of 41 μm. All samples were prepared for three runs of *in situ* dairy incubation.

Table 3.1. Lactation characteristics of milking cows in the *in situ* study

# of animal	Stock #	DIM	Lactation period	Average production
1	939	212	3rd lactation	48 kg
2	957	153	3rd lactation	52 kg
3	975	151	3rd lactation	56 kg
4	996	307	2nd lactation	40 kg

Notes: DIM: days in milk. Average production per day.

Procedure:

Rumen incubations were performed at 0, 2, 4, 8, 12, and 24 h; two bags were used per each time point of 0, 2, 4, and 8 h; for 12 and 24 h four bags were included. Nylon bags were inserted sequentially according to times of incubation; a polyester mesh lingerie bag was used to hold the nylon bags in the rumen (30 maximum per cow) and a weight to keep the bags merged in rumen fluid. At the last point time all inserted bags were removed at once ("gradual addition/all out") (Yu et al. 2000).

After incubation, the bags were removed, and hand rinsed in a bucket with cold tap water to remove excess ruminal content and stop microbial activity; then, the bags were washed 6 times in a "grab-released" motion; after washing, the excess water was drained, and the bags were dried at 55° C for 48 h in a forced-air drying oven. Dried bags were weighed, and the residues were pooled according to treatments, incubation time and run. Residues were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) and stored in plastic vials for future chemical analyses of crude protein (CP LECO FP-528, AOAC 990.03), starch (AOAC 996.11, AACC 76.13, ICC standard method No. 168), and neutral detergent fiber – NDF (Van Soest et al, 1991 combined with Ankom A200 filter bag technique - Ankom Technology, Fairport, NY, USA). The disappearance of nutrients was calculated as the difference between the original samples and residue samples.

This procedure was performed using the first-order degradation kinetics model described by Ørskov and McDonald (1979) and Tamminga et al. (1994). Degradation parameters for dry matter, crude protein, carbohydrates and NDF were calculated using the following formula: $R(t) = U + D \times e^{-Kd\times(t-T0)}$; for starch: $R(t) = D \times e^{-Kd\times(t-T0)}$ where, R(t) = residue percentage at t hours of incubation in the rumen (%), U = undegradable fraction (%), D = potentially degradable fraction (%), Kd = degradation rate (%/h), and T0 = lag time (h).

The effectively degradable fractions (ED) or extent of degradation in the rumen, as well as the ruminally undegradable fractions (RU) of the nutrients were determined with equations described in NRC Dairy (2001) and Yu et al. (2003); $ED = S + D \times Kd / (Kp + Kd)$; $RU = U + D \times Kp / (Kp + Kd)$ where, S represents the soluble fraction (%), Kp is the flow of degraded food from the rumen, which was assumed to be equal to 6%/h (Tamminga et al. 1994).

uNDF determination:

This assay was performed following the procedure of Lopes et al. (2015).

3 g of previous ground samples were weighed in duplicates into 5 x 10 cm Ankom bags with 6 μm pore size and then exposed to 288 hours of incubation in the rumen. After removal, the bags were washed in cold tap water 6 times and then oven dried at 40 °C for 48 h. After drying, bags were weighed to record residue values and finally used for NDF analysis (Van Soest et al. 1991) and weighed again to obtained uNDF values.

3.5.6. Intestinal digestibility of feed nutrients using a three-step in vitro technique

Intestinal digestibility of feed nutrients was estimated by following the procedure described by Calsamiglia and Stern (1995) and modified by Gargallo et al. (2006):

0.3 g – 0.4 g of residual sample from 12 h rumen incubation for each treatment were weighed into a 50 ml centrifuge tube in duplicates. All solutions and samples were prepared the same morning of the analysis. 10 ml of pepsin (Sigma P-7012) solution 0.1 mol/L HCl (pH = 1.9) was added to every tube, vortexed, and incubated at 38 °C in a shaking water bath (Precision, Serial Nº: 602071249, Thermo Scientific, USA) for 1 h. This was followed by 0.5 ml 1 mol/L NaOH solution + 13.5 ml of pancreatin (Sigma P-7545; pH = 7.8) were added; the tubes were vortexed and incubated at 38 °C for 24 h. Tubes were vortexed three times during incubation.

After incubation, 3 ml of trichloroacetic acid (TCA) were added to stop the enzymatic hydrolysis, tubes were vortexed and maintained at room temperature for 15 min. Next, the tubes were centrifuged for 15 min at 5000 rpm. Later, the 5 ml of supernatant were analyzed for soluble N using the Kjeldahl method (AOAC 984.13).

Intestinal digestion of protein was obtained as TCA-soluble N divided by the amount of N in the rumen residual sample (Gargallo et al. 2006; Calsamiglia and Stern, 1995).

3.5.7. Hourly effective rumen degradation ratios and potential nitrogen to energy synchronization in the rumen

Rumen microbes use nitrogen (N) and energy sources for their growth; scientific studies have shown that values around 32 g N/kg CHO or 25 g N/kg OM truly fermented in the rumen were optimal for microbial growth (Sinclair et al. 1993; Tamminga et al. 2007; Huang et al. 2015). Levels above these values will increase the ammonia content in the rumen, as microbes will not capture extra N for microbial protein synthesis. The excess ammonia will be transformed in urea and then excreted in the urine (Tas et al. 2006). In order to decrease the N losses and maximize the microbial synthesis in the rumen, the effective degradability of N and energy should be considered. For the present study, the effective rumen degradation of N and carbohydrates (CHO) was obtained following the calculation from Sinclair et al. (1993): Hourly ED (g/kg DM) = S + $[(D \times K_d) / (K_p + K_d)] \times [1 - e^{-t \times (Kd + Kp)}]$. The hourly effective degradation ratios of N to CHO were computed following the next equations:

$$\text{Hourly ED ratio N/CHO}_t = 1000 \times (\text{HEDN}_t - \text{HEDN}_{t-1}) / [(\text{HEDNDF}_t - \text{HEDNDF}_{t-1}) + (\text{HEDNFC}_t - \text{HEDNFC}_{t-1})],$$

where N/CHO_t = N to CHO ratio at time t (g N/kg CHO); HEDN_t = hourly effective degradability of N at time t (g/kg DM); HEDN_{t-1} = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO_t = hourly effective degradability of CHO at time t (g/kg DM); HEDNDF_t = hourly effective degradability of neutral detergent fiber at time t (g/kg DM); HEDNDF_{t-1} = hourly effective degradability of neutral detergent fiber at 1 h before t (g/kg DM); HEDST_t = hourly effective degradability of starch at time t (g/kg DM); HEDST_{t-1} = hourly effective degradability of starch at 1 h before t (g/kg DM) (Yang et al. 2013; Sinclair et al. 1993; Tamminga et al. 1990).

3.5.8. Prediction of truly digestible protein supply to the small intestine in dairy cattle

3.5.8.1. DVE/OEB Dutch system

The prediction of protein supply in dairy cows was briefly described by Yu (2005c) based on Tamminga (1994):

- Rumen undegraded feed protein (BCP): BCP (g/kg DM) = 1.11 × CP × %RUP, where RUP = $U + D \times K_p / (K_p + K_d)$; K_p (passage rate) = 0.06 h^{-1}; 1.11 = regression coefficient of *in vivo* on *in situ* degradation data.
- Microbial protein synthesized in the rumen based on available energy (MREE): MREE (g/kg DM) = FOM (g/kg) × 0.15, where FOM: fermented organic matter in the rumen and factor 0.15 means 150 g of microbial protein is assumed to be synthesized per kg FOM.
- Truly digested rumen synthesized microbial protein (DVME): DVME (g/kg DM) = 0.85 × 0.75 × MREE, where 85% is the assumed digestibility of microbial protein; 75% is assumed to be presented as amino acids.
- Truly absorbed bypass feed protein in the small intestine (DVBE):

$$\text{DVBE (g/kg DM)} = \text{dRUP} \times \text{BCP}$$

- Endogenous protein loss in the small intestine (ENDP): ENDP (g/kg) = 0.075 × UDM, where UDM: amount of undigested DM excreted in feces estimated as undigested organic matter plus undigested ash. According to the DVE/OEB system, 75 g of absorbed protein per kg of undigested DM in fecal excretion is required to compensate for the endogenous losses.
- Truly digested and absorbed protein in the small intestine (DVE):

$$\text{DVE (g/kg DM)} = \text{DVME} + \text{DVBE} - \text{ENDP}$$

- Degraded protein balance (OEB): OEB = $MCP_{RDP} - MCP_{FOM}$, where MCP_{RDP}: microbial protein synthesis from rumen degradable protein; MCP_{FOM}: microbial protein synthesis from energy extracted during anaerobic fermentation in the rumen.

3.5.8.2. National Research Council (NRC) Dairy Model

Calculations were performed following the description in NRC 2001.

- Rumen undegraded feed protein (RUP): RUP = CP × RUP.
- Rumen degraded feed protein (RDP):

  RDP = CP × RDP, where RDP = $S + D \times K_d / (K_p + K_d)$; $K_p = 0.06\ h^{-1}$.

- Rumen microbial protein synthesis (MCP*):* MCP = 0.13 × TDN, where 0.13 factor means that 130 g of microbial protein is assumed to be synthesized per kg of TDN.
- Truly absorbed microbial protein (AMCP): AMCP = 0.80 × 0.80 × MCP^{NRC}, where 80% is the assumed digestibility and true protein available from MCP.
- Truly absorbed rumen undegraded feed protein in the small intestine (ARUP):

  ARUP = dRUP × RUP

- Rumen endogenous crude protein (ECP): ECP = 6.25 × 1.9 × DM (g/kg)/1000.
- Truly absorbed endogenous protein in the small intestine (AECP): AECP = 0.50 × 0.80 × ECP, where 50% of rumen endogenous CP is assumed to pass to the duodenum; 80% of rumen endogenous CP is assumed to be true protein.
- Metabolizable protein (MP): accounts for digestible rumen undegraded feed protein, digestible rumen microbial protein synthesis, and rumen endogenous crude protein:

  MP = ARUP + AMCP + AECP

- Degraded protein balance (DPB): $DPB^{NRC} = RDP^{NRC} - 1.18\ MCP_{TDN}$, where RDP is the potential microbial protein synthesis based on rumen degraded feed protein and that based on 1.18 times energy (TDN) available for microbial fermentation in the rumen.

3.5.9. Statistical analyses

Results from chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios, intestinal digestibility of protein, predicted truly absorbed protein supply and feed milk value were analyzed using the Mixed model procedure of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US). RCBD was used as experimental design with the following model for analysis:

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

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 genotypes as a fixed effect (i= 1 to 8); β_j was the block effect of location (j= 1 to 3) and e_{ij} is the random error associated with the observation ij.

PROC NLIN-Gauss-Newton method of SAS was used to fit the rumen degradation data to the model. The differences among treatments was evaluated with a multiple comparison analysis using the Tukey method. SAS Contrast statements were performed to compare differences between low tannin faba bean and normal tannin faba bean. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$ but > 0.05.

3.6. Results and Discussion

3.6.1. Effect of genotypes on condensed tannins (CT) and basic nutrient profiles

Data obtained from the condensed tannin analysis of faba bean showed different ($P < 0.01$) condensed tannin content between low tannin genotypes (LT) and normal tannin genotypes (NT) ($P < 0.01$). The mean of CT in NT (Fatima, Vertigo, FB9_4, 346_10, and SSNS_1) was 7.8 times

higher than LT (Snowbird, Snowdrop, and 219_16) with an average of 3.9 % DM and 0.5 % DM, respectively. However, among the three LT and the five NT there was not a remarkable difference in the % of CT. Detailed results are expressed in Table 3.2. Specific data related to condensed tannin analysis in faba bean is hard to compare, as different analytical methods could be used to determine phenolic compounds. However, published information by Makkar et al. (1997) used most similar methods to this research; they found a CT content of 2.6 % in colored flowered faba bean while in white colored flowers, CT (as leucocyanidin equivalent) could not be detected.

The nutrient profiles of newly developed faba bean genotypes are presented in Table 3.3. Contrast results between LT and NT showed differences ($P < 0.05$) in the basic chemical, protein, carbohydrates, and fiber profiles. ($P < 0.05$). Organic matter (OM) was higher in NT than LT; Snowdrop, Fatima, Vertigo, 346_10, and SSNS_1 had a higher OM than Snowbird, 219_16, and FB9_4 but they were similar to Snowdrop and Fatima. The crude protein content was not significantly different ($P > 0.10$) between LT and NT; all faba bean genotypes showed a high CP mean around 28.3 %. In the present study the range of CP content was between 26.8 to 29.5 % DM, which was similar to results reported by Frejnagel et al. (1997) which obtained values of CP around 27.2 to 28.9 % DM. Gdala and Buraczewska (1997) found a CP range of 27.7 to 30.6 % DM in eight different cultivars of faba bean and concluded that crude protein was significantly affected by the different cultivars used in their study. Additionally, Garrido et al. (1991) performed a research in Canada where they found no difference between faba bean lines with white, normal, or diffused colored flowers. Results from soluble crude protein (SCP) showed a higher content in LT (20.9 % DM) and neutral detergent insoluble crude protein (NDICP, 2.2 % DM) compared to NT (SCP, 17.7 % DM; NDICP, 1.5 % DM); however, the three low tannin types were similar as

well as the five normal tannin types were not different between each other in SCP and NDICP content. The acid detergent insoluble crude protein (ADICP) was slightly higher in NT than LT.

The results related to carbohydrate profiles showed higher ($P < 0.05$) carbohydrate content in Snowbird, Fatima, Vertigo, and SSNS_1; although, Fatima and SSNS_1 were similar to all the other faba bean genotypes. LT showed a higher content of starch (37.4 % DM) and non-structural carbohydrates (NSC, 41.9 % DM) compared to NT (35.2 % DM and 39.5 % DM, respectively). Snowbird, Fatima, and Vertigo showed higher values for non-fiber carbohydrates (NFC) compared to the other genotypes and Fatima and Vertigo were similar to all six faba bean. Bhatty (1973) found that proteins and carbohydrates were the most variable fractions among 12 different faba bean cultivars and proved to be inversely related to each other. Faba bean carbohydrates include soluble starch, soluble polysaccharides, insoluble starch, free sugars, cellulose, hemicellulose and lignin (Bhatty, 1973). These inherent factors along with different methods of analysis could contribute to the final variation in CHO features.

The results for fiber content showed that NT had higher ($P < 0.05$) % of neutral detergent fiber (NDF, 18.4 % DM), acid detergent fiber (ADF, 11.1 % DM), and acid detergent lignin (ADL, 0.4 % DM) compared to LT (17.5 %, 10.7 %, and 0.3 % DM, respectively). Garrido et al. (1991) reported that ADL and ADF in normal flowered lines were higher than white flowered lines, suggesting a possible interference of phenolic components during the chemical analysis. Another study stated that the content of fiber varies proportionately to the dimension of the seed hull (Frejnagel et al. 1997). The average means of DM (92.8 %), EE (0.8 %), NPN (11.8 %), sugars (4.4 %), cellulose (10.6 %), and hemicellulose (7.1 %) were not significantly different ($P > 0.10$) among the 8 faba bean genotypes grown in western Canada.

Table 3.2. Condensed tannin content of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin varieties/lines (LT)			Normal tannin varieties/lines (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
CT (abs/mg)	0.01[b]	0.01[b]	0.01[b]	0.04[a]	0.05[a]	0.04[a]	0.04[a]	0.05[a]	0.005	<0.01	<0.01
CT (% DM)	0.4[b]	0.5[b]	0.5[b]	3.8[a]	4.2[a]	3.7[a]	3.8[a]	4.2[a]	0.43	<0.01	<0.01

Notes: [a-b] Means with different letters in the same row are significantly different (P < 0.05). CT, condensed tannins; SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. abs: absorbance units. Samples from 3 different locations as replications.

Table 3.3. Chemical profile of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Basic nutrient profile											
DM (%)	92.9	92.7	92.8	92.6	92.6	92.7	92.8	92.9	0.24	0.87	0.61
Ash (% DM)	3.3[a]	3.2[ab]	3.3[a]	3.2[ab]	3.1[b]	3.3[a]	3.1[b]	3.1[b]	0.12	0.04	0.02
EE (% DM)	0.9	0.7	1.0	0.9	0.9	0.7	0.7	0.7	0.09	0.20	0.16
FA (% DM)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.58	0.98
OM (% DM)	96.7[b]	96.8[ab]	96.7[b]	96.9[ab]	97.0[a]	96.7[b]	96.9[a]	97.0[a]	0.12	0.04	0.02
Protein profile											
CP (% DM)	27.1[bc]	28.7[abc]	28.5[abc]	28.3[abc]	26.8[c]	29.0[ab]	29.5[a]	28.6[abc]	0.58	<0.01	0.29
SCP (% DM)	20.3[ab]	21.1[a]	21.2[a]	17.6[c]	16.8[c]	19.0[abc]	18.1[bc]	17.2[c]	0.70	<0.01	<0.01
SCP (% CP)	74.8[a]	73.7[a]	74.5[a]	62.1[bc]	62.8[bc]	65.3[b]	61.1[bc]	60.1[c]	1.33	<0.01	<0.01
NPN (% DM)	11.4	10.4	12.1	12.2	12.2	11.4	12.8	11.6	1.72	0.97	0.51
NPN (% CP)	41.7	36.1	42.7	43.1	45.4	39.2	43.5	40.7	5.79	0.95	0.58
NDICP (% DM)	1.4[d]	1.6[bcd]	1.6[cd]	2.2[a]	2.1[abc]	2.1[ab]	2.3[a]	2.2[a]	0.14	<0.01	<0.01
NDICP (% CP)	5.1[c]	5.7[abc]	5.4[bc]	7.8[a]	7.9[a]	7.3[ab]	7.9[a]	7.7[a]	0.59	<0.01	<0.01
ADICP (% DM)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.28	0.02
ADICP (% CP)	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.12	0.38	0.04

Table 3.3. *Cont'd*. Chemical profile of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Carbohydrates profile											
CHO (% DM)	68.7^{ab}	67.4^{bc}	67.3^{bc}	67.7^{abc}	69.3^{a}	66.9^{bc}	66.7^{c}	67.7^{abc}	0.6	<0.01	0.67
Starch (% DM)	36.8	38.6	36.8	35.8	35.9	34.3	35.4	34.5	1.03	0.15	0.01
Starch (% NFC)	69.3	75.4	71.9	68.5	68.5	67.6	70.0	67.6	2.18	0.25	0.03
Sugar (% DM)	4.7	4.6	4.2	3.8	4.3	4.6	4.9	4.2	0.30	0.28	0.55
Sugar (% NFC)	8.8	8.9	8.2	7.2	8.1	9.0	9.6	8.2	0.61	0.24	0.66
NFC (% DM)	53.1^{a}	51.2^{b}	51.1^{b}	52.3^{ab}	52.5^{ab}	50.7^{b}	50.7^{b}	51.0^{b}	0.66	<0.01	0.18
NFC (% CHO)	77.3	78.0	76.0	77.2	75.7	75.8	76.0	75.4	0.66	0.15	0.26
NSC (% DM)	41.5	43.1	41.0	39.5	40.2	38.8	40.3	38.7	1.12	0.17	0.01
Fiber profile											
NDF (% DM)	17.0	17.8	17.7	17.7	19.0	18.4	18.3	18.9	0.53	0.06	0.01
ADF (% DM)	10.6	10.8	10.6	11.0	11.2	11.4	11.0	11.2	0.27	0.36	0.03
ADF (% NDF)	62.6	60.8	59.9	62.2	58.9	62.0	59.3	59.5	1.71	0.59	0.56
ADL (% DM)	0.4	0.2	0.2	0.5	0.5	0.4	0.4	0.4	0.08	0.10	0.01
ADL (% NDF)	2.1	1.0	1.1	2.6	2.8	2.2	2.1	2.3	0.45	0.13	0.01
NDFn (% DM)	15.6	16.2	16.1	15.5	16.9	16.2	16.0	16.6	0.46	0.21	0.34
Hemicellulose (% DM)	6.4	7.0	7.1	6.7	7.8	7.0	7.5	7.7	0.47	0.26	0.12
Cellulose (% DM)	10.2	10.6	10.4	10.5	10.7	11.0	10.5	10.8	0.24	0.51	0.16

Notes: $^{a-d}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparison using Tukey method. DM: dry matter; EE: ether extract (crude fat); CP: crude protein; OM: organic matter; FA: fatty acids; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrate. Samples from 3 different locations as replications.

3.6.2. Effect of genotypes and tannin levels on protein and carbohydrate sub-fractions

Protein fractions: PA2, PB1, PB2, PC and carbohydrate fractions: CA4, CB1, CB2, CB3, and CC were obtained using the Cornell Net Carbohydrate and Protein System (CNCPS 6.5). Results presented in Table 3.4. showed that the effect of genotypes and tannin levels on protein fractions was significant ($P < 0.05$). The rapidly degradable protein fraction (PA2) was higher in LT (+ 13.4 % of CP) compared to NT; the highest values were observed in all three LT. On the other hand, NT showed higher values of the moderately degradable protein fraction (PB1) (+ 9.8 % of CP), slowly degradable protein fraction (PB2) (+ 2.2 % of CP), and indigestible protein (PC) (+ 0.1 % of CP) compared to LT. In terms of carbohydrate fractions, the intermediately degradable carbohydrate fraction (CB1) was the only parameter that showed a significant difference ($P < 0.05$) with a higher content in LT (+ 3.2 % of CHO) compared to NT. The CNCPS feed library of NDS NDS professional ration formulation software (Version 3, RUM&N, NDS Professional, Reggio Nell'Emilia, Emilia-Romagna, Italy) provides protein and carbohydrate sub-fractions of white flowered faba bean, PB1 (22.3 % CP), CA4 (5.0 % CHO), CB1 (56.0 % CHO), and CB3 (24.9 % CHO) similar to the ones related to LT in the present study. On the other hand, NDS reported slightly lower values for PA2 (68.7 %), PB2 (3.3 %) but CHO fractions CB2 and CC disagreed with NDS library, showing highly different results in this research (CB2: 6.1 vs. 14.7 % CHO; CC: 7.9 vs. 0.9 % CHO).

Table 3.4. Protein and carbohydrate sub-fractions, degradable and bypass fractions of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Protein sub-fractions											
PA2 (% CP)	74.8^{a}	73.7^{a}	74.5^{a}	62.1^{bc}	62.8^{bc}	65.3^{b}	61.1^{bc}	60.1^{c}	1.33	<0.01	<0.01
PB1 (% CP)	20.1^{b}	20.6^{b}	20.0^{b}	30.1^{a}	29.3^{a}	27.4^{a}	31.0^{a}	32.2^{a}	1.04	<0.01	<0.01
PB2 (% CP)	5.0^{c}	5.7^{abc}	5.4^{bc}	7.7^{a}	7.8^{a}	7.2^{ab}	7.7^{a}	7.6^{a}	0.59	<0.01	<0.01
PC (% CP)	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.05	0.38	0.04
PA2 (% tp)	74.9^{a}	73.7^{a}	74.6^{a}	62.2^{bc}	62.9^{bc}	65.4^{b}	61.2^{bc}	60.3^{c}	1.33	<0.01	<0.01
PB1 (% tp)	20.1^{b}	20.6^{b}	20.0^{b}	30.1^{a}	29.4^{a}	27.4^{a}	31.1^{a}	32.2^{a}	1.04	<0.01	<0.01
PB2 (% tp)	5.0^{c}	5.7^{abc}	5.4^{bc}	7.7^{a}	7.8^{a}	7.2^{ab}	7.7^{a}	7.6^{a}	0.59	<0.01	<0.01
Carbohydrate sub-fractions											
CA4 (% CHO)	6.8	6.8	6.2	5.6	6.1	6.8	7.3	6.2	0.45	0.24	0.58
CB1 (% CHO)	53.6	57.2	54.7	52.8	51.8	51.2	53.1	51.0	1.42	0.11	0.01
CB2 (% CHO)	17.0	12.02	15.15	18.79	17.74	17.79	15.56	18.23	1.94	0.31	0.06
CB3 (% CHO)	21.3	23.37	23.23	21.07	23.41	23.51	22.73	22.56	0.90	0.24	0.99
CC (% CHO)	1.4	0.65	0.74	1.75	0.92	0.74	1.25	2.05	0.51	0.45	0.27

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different (P<0.05). PA2 = rapidly degradable protein fraction; PB1 = moderately degradable protein fraction; PB2 = slowly degradable protein fraction; PC = indigestible or unavailable protein fraction; tp = true protein; CA4 = rapidly degradable carbohydrate fraction (sugar); CB1 = intermediately degradable carbohydrate fraction (starch); CB2 = intermediately degradable carbohydrate fraction (soluble fiber); CB3 = slowly degradable carbohydrate fraction (digestible fiber); CC = unavailable neutral detergent fiber (indigestible fiber; SEM, Standard Error of Mean. Multi-treatment comparison using Tukey method. Samples from 3 different locations as replications; year 2016.

3.6.3. Effect of genotypes and tannin levels on energy values

The principal carbohydrate storage in the seed of legumes is represented by starch (around 22 to 45 %) (Hoover and Sosulski, 1991). Faba bean is a source of starch, some of the seed starch can bypass the rumen and undergo digestion later in the small intestine. Faba bean energy values are comparable with cereal grains such as barley. Fiber and oil contents are fairly low, however there is a high level of linoleic and linolenic acids in the seeds (O'Kiely et al. 2017).

According to results from energy values of different genotypes of faba bean (Table 3.5.), Snowbird, Fatima, and Vertigo had higher content of truly digestible non-fiber carbohydrates (tdNFC) compared to other genotypes, but Fatima and Vertigo was similar to all of them. 346_10, Snowdrop, 219_16, Fatima, FB9_4, and SSNS_1 showed higher levels of truly digestible crude protein (tdCP) than Snowbird and Vertigo. Results related to total digestible nutrients (TDN_{1x}), digestible energy (DE_{1x}), metabolizable energy (ME), net energy for lactation (NE_{Lp3x}), net energy for maintenance (NE_m), and net energy for gain (NE_g) were not significantly different ($P > 0.10$) among all different faba bean genotypes. Yu (2005b) reported a TDN_{1x} value of 81.1 % DM, ME-dairy (3.01 Mcal/kg), and NE_L (1.92 Mcal/kg) in faba bean. NDS library indicates that the TDN_{1x} content of white flowered faba bean is 78.5 % DM; these outcomes were not in agreement with the higher energy profiles obtained in the current research with faba bean that were grown in western Canada.

Table 3.5. Energy values of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Truly Digestible Nutrients (% DM)											
tdNFC	54.1^{a}	52.2^{b}	52.1^{b}	53.3^{ab}	53.5^{ab}	51.7^{b}	51.7^{b}	52.0^{b}	0.67	0.00	0.18
tdCP	27.1^{bc}	28.7^{abc}	28.5^{abc}	28.3^{abc}	26.7^{c}	29.0^{ab}	29.5^{a}	28.6^{abc}	0.58	0.01	0.31
tdFA	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.58	0.98
tdNDF	10.5	11.41	11.32	10.19	11.09	10.90	10.7	11.11	0.37	0.13	0.22
Total Digestible Nutrients (% DM)											
TDN_{1x}	84.8	85.3	85.0	84.7	84.4	84.6	84.9	84.7	0.28	0.53	0.11
Energy values (Mcal/kg)											
DE_{1x}	3.93	3.98	3.96	3.95	3.91	3.95	3.97	3.95	0.016	0.13	0.30
ME - dairy	3.20	3.24	3.22	3.21	3.18	3.21	3.23	3.22	0.014	0.14	0.31
NE_{L3X}	2.06	2.09	2.08	2.07	2.04	2.07	2.08	2.07	0.011	0.20	0.38
ME - beef	3.22	3.26	3.25	3.24	3.21	3.24	3.26	3.24	0.013	0.14	0.55
NE_m	2.22	2.24	2.23	2.23	2.20	2.23	2.24	2.22	0.010	0.21	0.40
NE_g	1.53	1.55	1.55	1.54	1.52	1.54	1.55	1.54	0.009	0.21	0.36

Notes: $^{a-c}$ Means with different letters in the same row are significantly different ($P<0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Values based on NRC-2001 Dairy and NRC-1996 Beef. tdNFC, truly digestible non-fiber carbohydrate; tdCP: truly digestible crude protein; tdFA: truly digestible fatty acid; tdNDF: truly digestible neutral detergent fiber. $TDN_{1\times}$: total digestible nutrient at one time maintenance. $DE_{1\times}$: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for growth. Samples from 3 different locations as replications; year 2016.

3.6.4. Effect of genotypes and tannin levels on in situ rumen degradation of dry matter

Degradation kinetic parameters of dry matter (DM) of different genotypes of faba bean are presented in Table 3.6. In terms of DM degradation rate (Kd), LT had a higher Kd compared to NT (9.05 %/h vs. 7.79 %/h). Snowbird showed a higher Kd value (10.12 %/h) compared to SSNS_1 (6.66 %/h). However, these two were not different from other genotypes. The soluble fraction in the rumen (S) was also higher in LT (32.9 % vs. 30.7 %). 346_10 and SSNS_1 showed lower values than Snowbird, but the three were similar to the other faba bean genotypes. Results of rumen undegradable dry matter (BDM) showed that NT were higher (+ 3 %) compared to LT; SSNS_1 had a higher content of BDM (34.1 %) than Snowbird (25.8 %), but both were similar to the other samples. The results reported by González and Andrés (2003) disagreed with the present study as lower content for S, D and EDDM in both low and high tannin faba bean types was observed. The previous published Kd value in low tannin types agreed with the current results (9.05 vs. 8.84 %/h) but was higher for the tannin type compared to the NT in this study (7.79 vs. 9.46 %/h).

3.6.5. Effect of genotypes and tannin levels on in situ rumen degradation of crude protein

Degradation results for crude protein (CP) are shown in Table 3.7. Degradation parameters related to rate of degradation (Kd) and degraded crude protein in the rumen ($EDCP_{DVE}$, RDP_{NRC}) were not significantly different ($P > 0.10$) among the faba bean varieties; rumen undegraded protein (BCP_{DVE}, RUP_{NRC}) was not different either but data showed a tendency to be significant ($P \leq 0.10$). Differences were observed in the soluble (S) and degradable (D) fractions of crude protein; Snowbird presented a higher S fraction (43.2 %) and lower D fraction (56.8 %) than 346_10 and SSNS_1; however, these varieties were similar to the others in both parameters. These results did not agree with the data reported by Goelema et al. (1998) which found a greater S fraction (+ 29.2

%) and lower D fraction, Kd, and RUP values (- 29.1 %, - 6.5 %/h, and - 2.9 %, respectively). González and Andrés (2003) published research agreed with the results of this present study in terms of Kd, S fraction, D fraction and EDCP. The variability between studies could be related to the sample type or cultivar and the difference of CP content as well as protein structure make-up conformation in the faba beans used.

3.6.6. Effect of genotypes and tannin levels on in situ rumen degradation of starch

Table 3.8 shows data obtained for degradation parameters of starch according to the DVE/OEB model (Tamminga et al. 1994). The majority of results were not significantly different ($P > 0.10$) among faba bean genotypes. A higher level of undegraded starch content (% BST) was observed in SSNS_1 (26.4 %) compared to 346_10 (17.9 %); however, these results were not different from Snowbird, Snowdrop, Vertigo, and FB9_4. The effective degraded starch in the rumen (% EDST) was different between 346_10 and SSNS_1 and presented similar values among the other genotypes. The content of undegraded starch (BST g/kg DM) was higher in LT (+ 9) compared to NT.

Starch degradation characteristics in the present research disagreed with previous studies published by Goelema et al. (1998) where higher values for S fraction and BST (g/kg DM) and lower Kd among faba bean were reported. However, present data demonstrated the high solubility and degradability of starch in the rumen of faba bean genotypes grown in western Canada which agreed with Yu (2005b).

Table 3.6. Degradation kinetics of dry matter (DM) of different genotypes of faba bean seeds with low and high tannin levels grown in western Canada.

Item	Low tannin genotypes (LT)			Normal tannin genotypes (NT)					SEM	P value	Contrast P value
	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1			LT vs. NT
Dry matter degradation											
Kd (%/h)	10.12^{a}	8.58^{ab}	8.46^{ab}	7.54^{ab}	8.16^{ab}	7.70^{ab}	8.88^{ab}	6.66^{b}	0.644	0.04	0.01
T0 (h)	3.8	1.7	2.2	3.5	3.5	2.4	2.4	1.7	0.53	0.05	0.66
Residue (0 h, %)	63.4^{b}	68.4^{ab}	69.6^{ab}	67.1^{ab}	69.0^{ab}	67.7^{ab}	71.1^{a}	71.76^{a}	1.44	0.02	0.05
S (%)	36.7^{a}	31.6^{ab}	30.8^{ab}	32.9^{ab}	31.0^{ab}	32.3^{ab}	28.9^{b}	28.2^{b}	1.44	0.02	0.05
D (%)	60.4	68.2	69.6	67.1	69.0	67.7	68.5	71.8	2.38	0.13	0.14
U (%)	2.9	0.2	0.0	0.0	0.0	0.0	2.6	0.00	1.34	0.57	0.62
% BDM = % RUDM	25.8^{b}	28.4^{ab}	29.0 ^{ab}	29.7^{ab}	29.3^{ab}	29.7^{ab}	30.8^{ab}	34.1^{a}	1.22	0.01	<0.01
% EDDM	74.3^{a}	71.6^{ab}	71.0^{ab}	70.3^{ab}	70.7^{ab}	70.3^{ab}	69.2^{ab}	65.9^{b}	1.22	0.01	<0.01

Notes: $^{a-b}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter. Samples from 3 different locations as replications; year 2016.

Table 3.7. Degradation kinetics of crude protein (CP) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Crude protein degradation											
CP (g/kg DM)	271^{bc}	287^{abc}	285^{abc}	283^{abc}	268^{c}	290^{ab}	295^{a}	286^{abc}	5.8	<0.01	0.29
Kd (%/h)	10.57	10.41	10.80	10.16	9.95	10.26	11.37	9.24	0.731	0.59	0.45
T0 (h)	2.7	2.4	2.2	4.0	3.5	3.1	3.1	2.8	0.40	0.13	0.01
Residue (0 h, %)	56.8^{b}	62.8^{ab}	62.7^{ab}	59.6^{ab}	62.3^{ab}	60.7^{ab}	65.6^{a}	66.4^{a}	2.10	<0.01	0.05
S (%)	43.2^{a}	37.2^{ab}	37.3^{ab}	40.4^{ab}	37.7^{ab}	39.3^{ab}	34.5^{b}	33.6^{b}	2.10	<0.01	0.05
D (%)	56.8^{b}	62.8^{ab}	62.7^{ab}	59.6^{ab}	62.3^{ab}	60.7^{ab}	65.5^{a}	66.4^{a}	210	0.01	0.05
% BCP = % RUP	20.6	23.0	22.4	22.2	23.5	22.5	23.1	26.2	1.33	0.22	0.13
BCP (g/kg DM, DVE)	62	73	71	70	70	72	76	83	4.8	0.10	0.09
RUP (g/kg DM, NRC)	56	66	64	63	63	65	68	75	4.4	0.10	0.09
% EDCP = % RDP	79.4	76.9	77.6	77.8	76.6	77.5	76.9	73.8	1.33	0.22	0.13
EDCP=RDP (g/kg DM)	215	221	221	220	205	225	227	211	5.4	0.14	0.67

Notes: $^{a-c}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. CP: crude protein; Kd: rate of degradation of D fraction (%/h); D: potentially degradable fraction; T0: lag time; S: soluble fraction in the in situ incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein; RDP: rumen degradable protein. Samples from 3 different locations as replications; year 2016.

Table 3.8. Degradation kinetics of starch (ST) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Low tannin varieties/lines (LT)			Normal tannin varieties/lines (NT)					SEM	P value	Contrast P value
	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1			LT vs. NT
Starch degradation											
ST (g/kg DM)	368	386	368	358	359	343	354	345	10.3	0.15	0.01
Kd (%/h)	13.76	13.48	11.38	14.65	13.02	13.74	15.55	10.63	0.980	0.05	0.38
T0 (h)	5.7	5.0	4.1	6.8	6.2	5.4	6.1	4.7	0.78	0.31	0.14
Residue (0 h, %)	67.5	68.5	73.1	67.7	71.0	67.6	64.4	73.1	3.08	0.42	0.68
S (%)	32.5	31.5	26.9	32.5	29.0	32.4	35.6	26.9	3.08	0.42	0.68
D (%)	67.5	68.5	73.1	67.7	71.0	67.6	64.4	73.1	3.08	0.42	0.68
%BSt	20.5^{abc}	21.3^{abc}	25.2^{ab}	19.7^{bc}	22.7^{abc}	20.8^{abc}	17.9^{c}	26.4^{a}	1.38	0.01	0.38
BSt (g/kg DM)	84^{bc}	91^{ab}	103^{a}	78^{bc}	90^{ab}	79^{bc}	70^{c}	101^{a}	5.2	<0.01	0.03
%EDSt	79.5^{abc}	78.7^{abc}	74.8^{bc}	80.3^{ab}	77.3^{abc}	79.2^{abc}	82.1^{a}	73.6^{c}	1.38	0.01	0.38
EDST (g/kg DM)	293	304	275	287	278	272	291	254	11.2	0.13	0.10

Notes: $^{a-c}$ Means with different letters in the same row are significantly different ($P<0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. ST: starch; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch. Samples from 3 different locations as replications; year 2016.

3.6.7. Effect of genotypes and tannin level on in situ rumen degradation of neutral detergent fiber

Results of rumen degradation kinetics of neutral detergent fiber (NDF) are presented in Table 3.9. Data showed no difference ($P > 0.10$) in the rate of degradation (Kd) of NDF, effective degraded NDF (% EDNDF, EDNDF g/kg), and uNDF content among different genotypes of faba bean. The degradable fraction (D) of NDF in the rumen was higher ($P < 0.05$) in LT (+ 16.2 %); however, the undegradable fraction (U) was not different ($P > 0.10$) between LT and. NT. Fatima and Vertigo, however, showed the lower ($P < 0.05$) U fraction level compare to 346_10. The rumen undegradable neutral detergent fiber (RUNDF) was lower ($P < 0.05$) in 219_16 than SSNS_1, but both were not different from the other genotypes. In terms of LT versus NT types, RUNDF was lower in LT ($P < 0.01$).

3.6.8. Effect of genotypes and tannin levels on intestinal digestibility of feed nutrients

The intestinal digestibility and total tract digestion of DM and CP of different genotypes of faba bean are presented in Table 3.10. Parameters related to dry matter digestion showed no significant difference ($P < 0.05$) among genotypes, however a tendency ($P \leq 0.10$) was observed in the intestinal digestible rumen bypass DM (IDBDM) and total digestible DM (TDDM). In terms of crude protein digestion parameters, LT showed higher intestinal digestibility of rumen bypass protein (dIDP) (+ 8.0 %) compared to NT. Snowdrop and 219_16 had higher dIDP (83.5 and 83.3 %, respectively) than FB9_4 and SSNS_1 (74.1 and 74.6 %, respectively); however, the low tannin genotypes were not different between each other as well as the normal tannin genotypes were similar among each other. Data related to intestinal absorbable feed protein (IADP) was not significantly different ($P > 0.10$) among the genotypes. Snowdrop, 219_16, and 346_10 were higher (276, 274, and 278 g/kg DM) than Vertigo (253 g/kg DM) in terms of total digestible protein (TDP), but these genotypes were similar the other faba bean.

Table 3.11. presents the results of intestinal digestibility and total tract digestion of starch and neutral detergent fiber (NDF) of different genotypes of faba bean grown in western Canada. Data showed higher levels of intestinal digestibility of rumen bypass starch (dBST) in NT (+ 10.7 %) compared to LT, resulting on a higher % of total digestible starch (TDST) in NT (+ 2.1 %) compared to LT. Fiber digestion parameters were not significantly different ($P > 0.10$), except in the total digestible NDF where, LT had a higher content (+ 4.8 %) compared to NT.

3.6.9. Effect of genotypes and tannin levels on hourly effective rumen degradation ratios and potential nitrogen to energy synchronization in the rumen

One objective of ruminant nutrition is to reach the optimal microbial synthesis with less nitrogen loss in the rumen to provide an adequate protein supply to dairy cows. Tamminga et al. (1994, 2007) and Sinclair et al. (1993) imply that the ideal proportion of effective degradable N to energy is 25 g of available N per kg of available OM or 32 g of available N per kg of available CHO. When these values are lower it could implicate a shortage of N (source for microbial growth); conversely, when increased this could indicate a potential N loss from the rumen with less energy available to rumen microbes (Nuez-Ortín and Yu, 2010).

As shown in Table 3.12, no significant differences ($P > 0.10$) were observed in ratios of available nitrogen to available carbohydrates (N/CHO), effective degradable nitrogen to effective degradable carbohydrate content (ED_N/ED_CHO), or effective degradation at h0, h1, h2, h3, and h4. However, at h6, h8, h10, h12, h16 greater results were observed in NT ($P < 0.05$) than LT. Higher values were found in FB9_4, 346_10, and SSNS_1 compared to the other genotypes as shown in Figures 3.1 and 3.2.

Table 3.9. Degradation kinetics of fiber (NDF) and indigestible fiber ($uNDF_{288h}$) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Fiber degradation											
NDF (g/kg DM)	170	178	177	177	190	183	183	189	5.3	0.06	0.01
Kd (%/h)	9.04	5.68	12.50	4.09	6.85	11.29	13.74	10.41	2.544	0.16	0.92
T0 (h)	1.1	0.0	0.1	0.7	0.9	2.5	0.0	0.0	0.97	0.65	0.87
Residue (0 h, %)	94.7	93.8	94.8	92.8	86.4	83.5	84.4	91.3	6.13	0.43	0.06
S (%)	5.27	6.23	5.23	7.16	13.64	16.47	15.57	8.74	6.13	0.43	0.06
D (%)	66.6^{abc}	74.5^{ab}	71.5^{ab}	84.2^{a}	67.9^{ab}	37.9^{cd}	37.1^{d}	46.0^{bcd}	9.75	0.02	0.04
U (%)	28.7^{abc}	19.3^{bc}	23.3^{abc}	8.7^{c}	18.5^{c}	45.7^{ab}	47.3^{a}	45.2^{ab}	8.91	0.04	0.16
% BNDF=% RUNDF	59.8	58.3	52.2	59.5	57.0	58.9	58.5	62.4	1.80	0.05	0.08
RUNDF (g/kg DM, NRC)	101^{ab}	104^{ab}	92^{b}	105^{ab}	108^{ab}	108^{ab}	107^{ab}	118a	4.0	0.02	<0.01
% EDNDF=% RDNDF	40.2	41.7	47.8	40.6	42.9	41.2	41.5	37.6	1.80	0.05	0.08
EDNDF=RDNDF (g/kg DM)	68	75	85	72	82	76	76	71	4.3	0.13	0.83
uNDF288h (% DM)	0.9	0.4	0.5	1.2	0.6	0.5	0.8	1.4	0.35	0.46	0.28

Notes: $^{a-d}$ Means with different letters in the same row are significantly different ($P<0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. NDF: neutral detergent fiber; Kd: the degradation rate of D fraction; T0: lag time; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. uNDF: undigestible neutral detergent fiber at 288 h based on CNCPS 6.5). Samples from 3 different locations as replications; year 2016.

Table 3.10. Intestinal digestibility and total tract digestion (DM and CP) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Dry matter digestion											
% dBDM	56.7	62.8	68.0	62.4	64.2	62.7	59.3	57.2	3.38	0.34	0.60
% IDBDM	14.6	17.8	19.7	18.5	18.8	18.6	18.3	19.5	1.16	0.10	0.10
IDBDM (g/kg DM)	146	178	197	185	188	186	183	195	11.6	0.10	0.10
% TDDM	88.8	89.5	90.7	88.8	89.5	88.9	87.5	85.4	1.09	0.10	0.06
TDDM (g/kg DM)	888	895	907	888	895	889	875	854	10.9	0.10	0.06
Crude protein digestion											
% dIDP	82.5^{ab}	83.5^{a}	83.3^{a}	75.4^{bc}	76.2^{abc}	74.1^{c}	75.0^{bc}	74.6^{c}	2.16	<0.01	<0.01
IADP (g/kg DM)	46	55	53	47	48	48	52	56	3.7	0.20	0.58
IADP (g/kg CP)	170	192	186	167	179	166	174	196	11.2	0.34	0.41
TDP (g/kg DM)	261^{ab}	276^{a}	274^{a}	267^{ab}	253^{b}	273^{ab}	278^{a}	267^{ab}	5.9	0.01	0.35
TDP (g/kg CP)	964^{a}	962^{ab}	962^{ab}	945^{abc}	944^{abc}	941^{bc}	943^{abc}	934^{c}	5.9	<0.01	<0.01
% IADP (% CP)	16.9	19.2	18.6	16.7	17.9	16.6	17.4	19.6	1.11	0.34	0.41
% TDP (% CP)	96.4^{a}	96.2^{a}	96.2^{a}	94.5^{ab}	94.4^{ab}	94.1^{ab}	94.3^{ab}	93.4^{b}	0.59	<0.01	<0.01

Notes: $^{a-c}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digestible rumen bypass protein; TDP: total digested crude protein. Samples from 3 different locations as replications; year 2016.

Table 3.11. Intestinal digestibility and total tract digestion (Starch and NDF) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Low tannin genotypes (LT)			Normal tannin genotypes (NT)					SEM	P value	Contrast P value
	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1			LT vs. NT
Starch digestion											
% dBST	77.8	80.5	89.3	95.0	97.9	98.1	92.7	82.5	6.45	0.22	0.04
% IDBST	16.3	17.4	22.5	18.7	22.3	20.4	16.7	21.7	2.18	0.28	0.45
IDBST (g/kg DM)	66	74	92	75	89	78	65	83	8.4	0.27	0.95
% TDST	95.7	96.1	97.3	99.1	99.7	99.6	98.7	95.4	1.22	0.10	0.03
TDST (g/kg DM)	359	378	367	362	367	349	356	337	9.9	0.22	0.08
Fiber digestion											
% dBNDF	22.7	29.3	24.2	19.9	24.4	18.4	16.0	22.9	3.66	0.33	0.08
% IDBNDF	13.7	17.0	12.5	11.8	13.9	11.1	9.3	14.4	2.19	0.38	0.17
IDBNDF (g/kg DM)	23	31	22	21	26	20	17	27	3.9	0.31	0.30
% TDNDF	53.8	58.7	60.4	52.4	56.9	52.2	50.8	51.9	2.47	0.11	0.02
TDNDF (g/kg DM)	91	105	107	93	108	96	93	98	5.3	0.16	0.34

Notes: Means with no letters were not significantly different (P>0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch. dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber. Samples from 3 different locations as replications; year 2016.

Table 3.12. Potentially available N to available CHO synchronization of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
N/CHO (g/kg)	81	81	84	85	78	88	88	84	3.4	0.26	0.26
ED_N/ED_CHO (g/kg)	91	87	93	90	86	101	97	97	5.2	0.40	0.32
Hourly effective degradation ratios of at individual times (g/kg)											
h0	154	133	180	148	131	144	122	125	35.3	0.86	0.32
h1	63	71	73	67	68	74	83	81	10.1	0.77	0.39
h2	64	71	72	68	69	76	85	82	9.5	0.63	0.27
h3	64	72	72	70	70	78	87	82	8.9	0.45	0.16
h4	65	72	72	71	70	80	90	83	8.5	0.26	0.08
h6	66^{b}	72^{ab}	72^{ab}	73^{ab}	71^{ab}	83^{ab}	94^{a}	84^{ab}	7.8	0.04	0.01
h8	67^{b}	72^{b}	71^{b}	74^{b}	72^{b}	87^{ab}	99^{a}	84^{ab}	7.6	0.01	<0.01
h10	67^{b}	73^{b}	71^{b}	74^{b}	72^{b}	91^{ab}	105^{a}	84^{ab}	7.9	0.01	<0.01
h12	67^{b}	69^{b}	70^{b}	72^{b}	72^{b}	94^{ab}	110^{a}	84^{ab}	8.8	0.01	0.01
h16	67^{bc}	63^{c}	69^{bc}	65^{c}	70^{bc}	101^{ab}	121^{a}	83^{abc}	12.1	0.03	0.03
h20	66	56	68	54	67	107	133	83	16.8	0.05	0.06
h24	67	46	68	42	64	111	144	83	22.2	0.08	0.11

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. ED: effective degradability; CHO: carbohydrates. Samples from 3 different locations as replications; year 2016

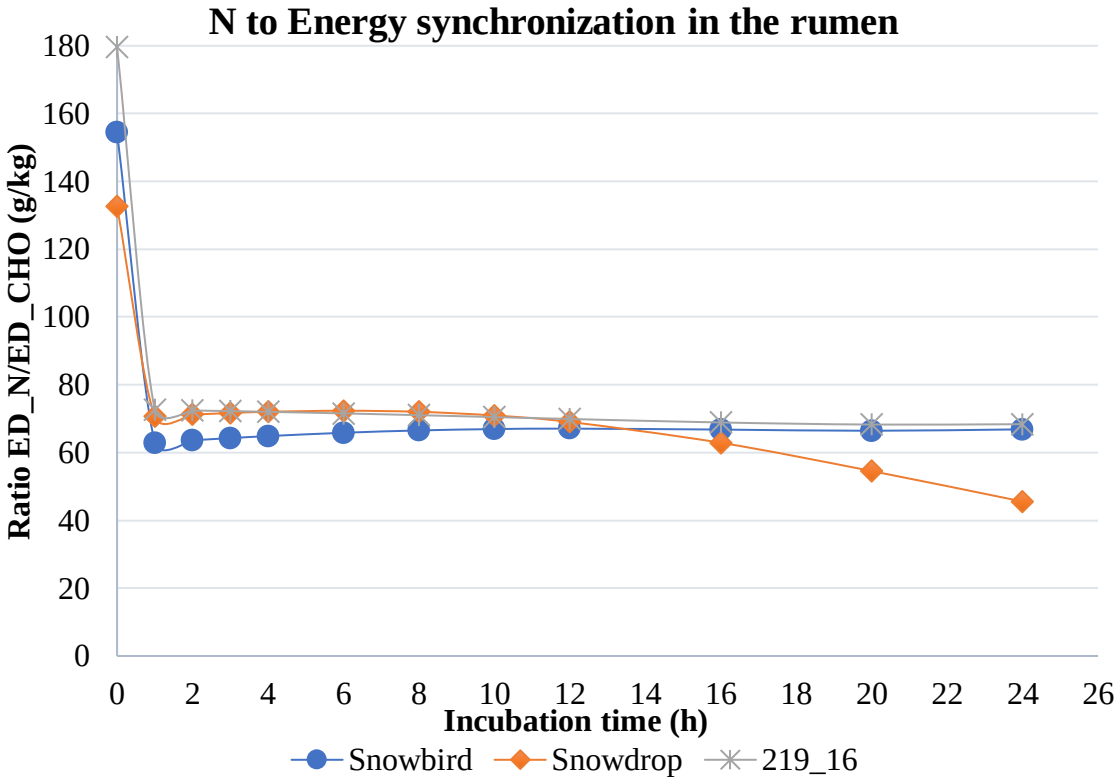


Figure 3.1. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of different genotypes of faba bean seeds with low tannin levels grown in western Canada.

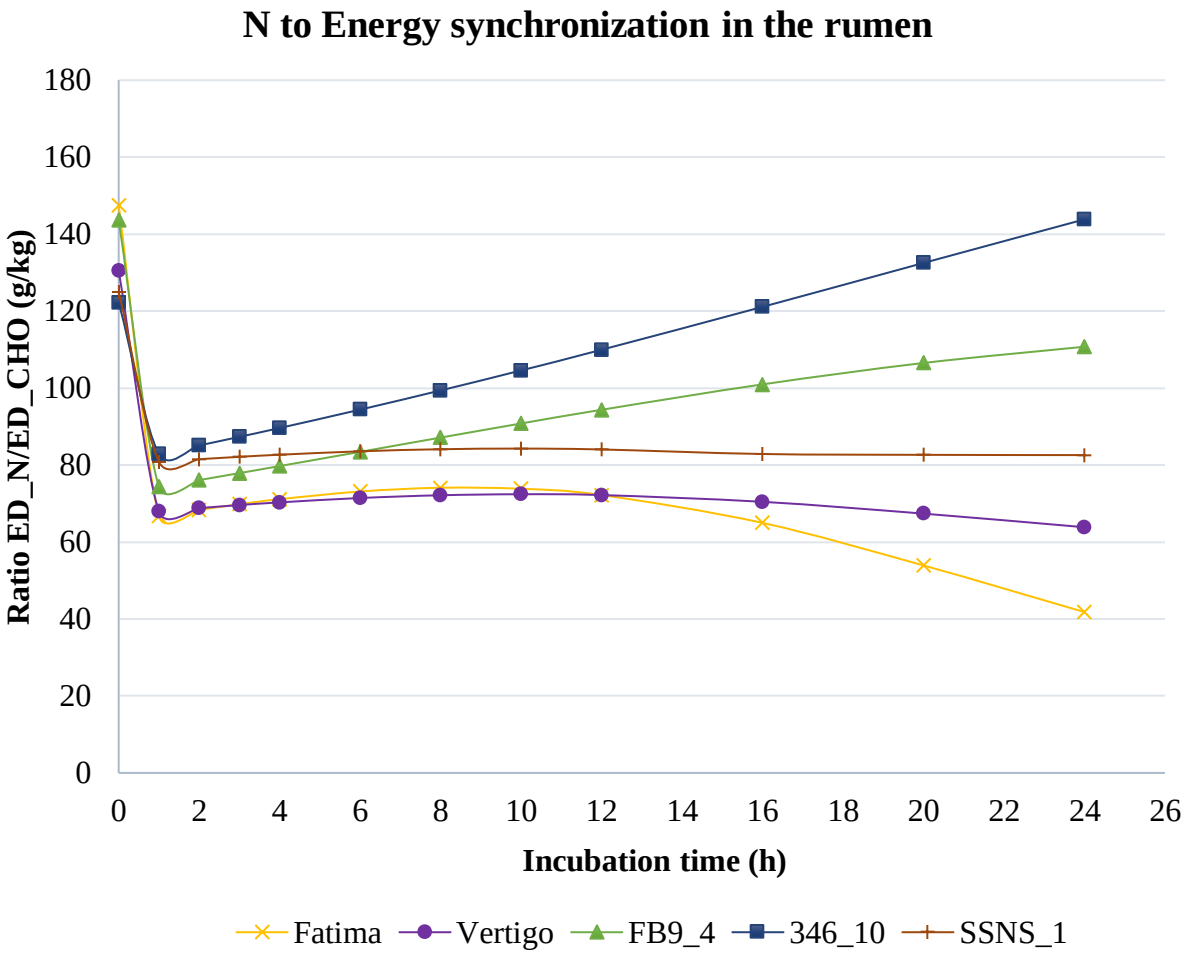


Figure 3.2. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of different genotypes of faba bean seeds with normal tannin levels grown in western Canada.

3.6.10. *Effect of genotypes and tannin levels on prediction of truly digestible protein supply to the small intestine in dairy cattle with the DVE/OEB system and NRC 2001 model.*

Results of metabolic characteristics and true nutrient supply based on the DVE/OEB system are presented in Table 3.13. The microbial protein synthesized in the rumen based on available energy (MREE) and the rumen synthesized microbial protein truly absorbed in the small intestine (DVME) was greater in Snowbird and 346_10 (+ 6 g/kg DM) compared to the lower value found in SSNS_1 but these results were similar to the other genotypes. Data related to the truly absorbed bypass protein in the small intestine (DVBE), the truly digested protein in the small intestine (DVE), the degradable protein balance (OEB), and feed milk value (FMV) was not significantly different ($P > 0.10$) among the eight genotypes of faba bean. In the case of rumen undegraded crude protein (BCP) a tendency ($P \leq 0.10$) was detected among genotypes and between LT and NT. The results from this study are different from published data by Yu et al. (2000), which obtained lower BCP and DVE values and a higher OEB in horse beans (*Vicia faba mayor*). A higher OEB value will indicate a potential N loss from the rumen due to an imbalance between nitrogen utilization and its degradation in the rumen.

No significant differences ($P < 0.10$) were shown in the metabolic characteristics and true nutrient supply based on the NRC 2001 model. Rumen undegradable crude protein (RUP) showed a tendency for significance with $P \leq 0.10$. Detailed results are presented in Table 3.14. Yu (2005b) reported lower values for RUP, ARUP, MP and DPB compared to the results observed in this study.

Table 3.13. Metabolic characteristics and truly absorbable nutrient supply (based on non-TDN system: DVE-OEB) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

	Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Truly digestible nutrient supply to dairy cows											
BCP (g/kg DM)	62	73	71	70	70	72	76	83	4.8	0.10	0.09
EDCP (g/kg DM)	215	221	221	220	205	225	227	211	5.4	0.14	0.67
MREE (g/kg DM)	123^{a}	120^{ab}	118^{ab}	122^{ab}	120^{ab}	121^{ab}	123^{a}	117^{b}	1.1	0.01	0.94
MREN (g/kg DM)	209	214	214	213	198	218	219	203	5.6	0.17	0.59
DVME (g/kg DM)	78^{a}	77^{ab}	75^{ab}	78^{ab}	76^{ab}	77^{ab}	78^{a}	74^{b}	0.7	0.01	0.94
DVBE (g/kg DM)	51	61	59	52	53	53	57	62	4.1	0.20	0.58
Degraded protein balance (OEB) and Total true protein supply (DVE) to dairy cows											
DVE (g/kg DM)	128	136	133	128	128	129	134	135	3.6	0.29	0.46
OEB (g/kg DM)	86	93	96	91	78	96	97	86	5.6	0.22	0.58
Feed Milk Value (kg milk/kg DM feed)											
FMV	2.60	2.77	2.70	2.61	2.60	2.62	2.72	2.74	0.073	0.28	0.45

Notes: $^{a-b}$ Means with different letters in the same row are significantly different ($P<0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. BCP: bypass crude protein; EDCP: effective degradability of CP; MREE: microbial protein synthesized in the rumen based on available energy; MREN: microbial protein synthesized in the rumen; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value. Samples from 3 different locations as replications; year 2016.

Table 3.14. Metabolic characteristics and true nutrient supply (based on TDN system: NRC dairy) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Low tannin genotypes (LT)			Normal tannin genotypes (NT)					SEM	P value	Contrast P value
	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1			LT vs. NT
Truly digestible nutrient supply to dairy cows											
RUP (g/kg DM)	56	66	64	63	63	65	68	75	4.4	0.10	0.09
MCP_{TDN} (g/kg DM)	101	102	101	101	101	101	101	101	1.1	0.52	0.11
MCP_{RDP} (g/kg DM)	183	188	188	187	174	191	193	179	4.6	0.14	0.67
AMCP (g/kg DM)	65	65	65	65	64	65	65	65	0.2	0.52	0.12
ARUP (g/kg DM)	46	55	53	47	48	48	52	56	3.7	0.20	0.58
ECP (g/kg DM)	11	11	11	11	11	11	11	11	0.0	0.87	0.52
AECP (g/kg DM)	4	4	4	4	4	4	4	4	0.0	0.82	0.60
Degraded protein balance (DPB) and Total metabolizable protein supply (MP) to dairy cows											
MP (g/kg DM)	115	125	122	116	117	117	121	125	3.7	0.17	0.51
DPB (g/kg DM)	96	101	101	101	86	106	107	92	5.4	0.14	0.76
Feed Milk Value (kg milk/kg DM feed)											
FMV	2.34	2.53	2.48	2.36	2.37	2.38	2.45	2.54	0.074	0.15	0.50

Notes: Means with no letters were not significantly different (P>0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. RUP: rumen undegradable feed crude protein; MCP_{TDN}: rumen synthesized microbial protein base on available TDN; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; AMCP truly absorbed microbial protein in the small intestine; ARUP: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value. Samples from 3 different locations as replications; year 2016.

3.7. Chapter summary and conclusions

Condensed tannin of normal tannin genotypes grown in western Canada was 3.5 % higher than the low tannin types. Chemical profiles were different among genotypes and between low and normal tannin types. Low tannin faba bean had higher SCP and starch while normal tannin presented greater OM, NDICP, ADICP, NDF, ADF, and ADL. The content of dry matter, crude protein, and carbohydrates of faba bean used in the present study was 92.8 %, 28.3 % DM, and 67.7 % DM, respectively. Despite the different chemical profiles among samples, no difference was observed in energy values. The seeds had a TDN_{1x} content of 84.8 % and NE_l of 2.07 Mcal/kg. The digestible protein and CHO sub-fractions were different with less PA2 and CB1 fractions and higher PB1, PB2 and PC fractions in normal tannin types compared to low tannin. Low tannin types presented a higher Kd for DM, higher degradable fraction (D) of NDF, so the EDDM was higher and RUNDF lower in these genotypes. CP degradation characteristics in the rumen were similar in both low and normal tannin types. The dIDP was larger in the low tannin types which also proved to have more TDP. Normal tannin genotypes had less BST content, but they showed a higher dBST and had more TDST and lower TDNDF (related to the lower degradability of NDF in the rumen).

The nutrient profiles of different genotypes of faba bean seeds grown in western Canada were different among varieties and between low and normal tannin types; however, the total nutrient supply (TDN, MP) to dairy cattle and the predicted production performance (FMV) was not significantly different. Low and normal tannin faba bean had different rumen degradation characteristics in terms of DM, starch, and NDF and different availability of CP and starch in the small intestine of dairy cattle. In general, faba bean grown in western Canada can be used as an alternative nutritive ingredient for ruminant diets, without a significant tannin or variety effect on metabolic characteristics and true nutrient supply.

4. EFFECT OF HEAT PROCESSING METHODS ON THE STRUCTURAL, PHYSICOCHEMICAL, AND NUTRITIONAL CHARACTERISTICS OF DIFFERENT GENOTYPES OF FABA BEAN WITH LOW AND NORMAL TANNIN LEVELS GROWN IN WESTERN CANADA.

4.1. Abstract

The purpose of this study was to determine the effects of heat processing methods, Steam Pressure (SP) and Microwave Irradiation (MI) on the nutrient rumen degradation and intestinal availability of faba bean grown in western Canada. Three low tannin and three normal tannin faba bean types were used. Half sample was subjected to Steam Pressure at 121°C for 30 min. and the other was microwaved for 3 min (900 W). Energy values were determined using NRC 2001. Rumen degradation kinetics using the standard *in situ* animal trial procedure with four cannulated cows following the 'gradual addition/all out' schedule at 0, 2, 4, 8, 12 and 24 hours. Intestinal digestion was determined using the three-step *in vitro* technique with 12 hours pre-rumen incubation sample. The experimental design was CRD with a 2 x 3 factorial treatment arrangement (2 tannin level x 3 treatments). Procedure of MIXED model of SAS 9.4 was used for statistical analyses with significance declared at $P < 0.05$. Steam Pressure and Microwave Irradiation increased ($P < 0.05$) the indigestible protein fraction (PC), intermediately degradable CHO fraction (CB1) and decreased the rapidly degradable CHO fraction (CA4) and $uNDF_{288h}$. Steam Pressure reduced ($P < 0.05$) the digestibility of rumen bypass starch (dBST) and total digested starch (TDST) while increased the intestinal digested crude protein (IADP) compared to the Control and Microwave Irradiation. The metabolizable protein (MP) and feed milk value (FMV) were higher with Steam Pressure treatment. Heat treatment affected differently the faba bean nutritional characteristics and changed the degradation kinetics of main nutrients (DM, CP and starch).

4.2. Introduction

Processing methods are used to change the physical, molecular and nutritional characteristics of different feedstuffs with the purpose of making feed ingredients more digestible and available to livestock animals.

In legumes, heat treatment can remove or inhibit antinutritional factors and improve the quality of the seeds. However, heat can affect feed nutrients in diverse forms. Altering the protein degradative behaviour with heat involves primarily denaturation of the protein secondary structure, this process relates to the organization of the general molecular structure of proteins by breaking hydrogen bridges and disulfide bonds (Salazar-Villanea et al. 2016). Heating methods result in protein structure stabilization and cross-linkages to carbohydrates. This effect protects proteins from ruminal hydrolysis, reducing their solubility and degradation rate in the rumen (Goelema et al. 1999). During the heating process, Maillard reactions can occur, causing microbial resistance of proteins to proteolysis; however, these reactions are reversible in early stages when the temperature is moderated. In this case, rumen degradability of protein can be reduced without negative effects on digestibility (Andrade-Montemayor et al. 2009).

Thermal treatments affect starch structure in different ways. First, swelling of granules will occur as a result of the exposure to water and gradual heat, up to (55^{o}C). After cooling and drying the swelling is reversible, but when more temperature is applied (60 to 80^{o}C), this process can become irreversible, causing the loss of crystallinity in the starch granules by gelatinization. Partial or complete gelatinization may occur depending on temperature, moisture content, and different processing times. Starch degradability in the rumen can be increased when steam is used during the heating process. Other effects of thermal treatment on starch is retrogradation, in which the molecules separated during gelatinization re-associate but without returning to its original form.

Retrograded starch can result in the formation of a less digestible fraction than native starch. (Andrade-Montemayor et al. 2009; Goelema et al. 1999).

The main purpose of heat treatment of ruminant feeds is to modify their carbohydrate degradation and decrease protein degradability to prevent nitrogen and energy losses in the rumen while improving microbial protein synthesis and increasing nutrient supply to the small intestine (Andrade-Montemayor et al. 2009; Yu et al. 2004).

4.3. Study objective

To determine the effects of different heat processing methods on the nutrient rumen degradation and intestinal availability of faba bean genotypes grown in western Canada.

4.4. Study hypotheses

1. Thermal treatments (Microwave Irradiation and Steam Pressure) will have different effects on physicochemical characteristics of different genotypes of faba bean.
2. Low tannin and normal tannin faba bean respond differently to heat processing methods resulting in different nutrient supply and availability for dairy cattle.

4.5. Materials and Methods

4.5.1. Ingredients and sample preparation

A total of 18 samples were analyzed in this study. Three faba bean genotypes with low tannin level (Snowbird, CDC Snowdrop, and CDC 219-16) and normal tannin level (CDC Fatima, 346-10, and CDC SSNS-1) were used for the present heat processing study. Each sample was subjected to two heating process (Microwave Irradiation and Steam Pressure Toasting), and raw samples were maintained as the Control treatment. The six genotypes of faba bean were taken from samples of the previous study. All samples were from the same location in Saskatchewan (Outlook) grown in 2016.

Samples were ground using a roller mill (Telemecanique. Emerson, Poland) with a roller gap of 0.508 mm before subjecting them to thermal treatments.

4.5.2. Processing methods

4.5.2.1. Microwave Irradiation: 300 g of ground samples were placed on a 100 mm watch glass and then heated for 3 min using a microwave oven model N° MO1900BC (Black & Decker, Miramar, FL, USA) with a power of 900 W and irradiation frequency of 2450 MHz. Time of heating was determined following the study by Yan et al. (2014).

4.5.2.2. Steam Pressure Toasting: 300 g of ground sample were placed on 3.8 cm by 12.7 cm aluminum drip pans and heated using a medium steam sterilizer (Amsco century, Steris). This process was performed on a 50 min. cycle which included 30 min. of sterilization at 121 °C, 10 min. of drying (vacuum dry at 10 inHg), and 10 min. for cooling down. The chosen treatment was based on previous studies performed by Yu et al. (2000). After the heat treatments samples were cooled to room temperature. Part of the sample was ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) to perform further chemical analyses and the rest was used for the rumen incubation and intestinal digestion study.

4.5.3. Determination of nutrient profiles

100 g of each sample were weighed and sent for basic chemical analyses at Cumberland Valley Analytical Services (CVAS, Waynesboro, PA, USA). Procedures followed by this laboratory are included below (CVAS, 2018):

- Dry matter: AOAC, 2000 (930.15)
- Ash: modified AOAC, 2000 (942.05) procedure using 1.5g sample and 4 h. ash time

- Acid detergent fiber (ADF)and lignin: AOAC, 2000 (973.18) modified procedure with whatman 934-AH glass micro-fiber filters with 1.5um particle retention used in place of fritted glass crucible for ADF only
- Crude Fiber: AOAC, 2000 (978.10)
- Fat: AOAC, 2006 (2003.05) Tecator Soxtec System HT 1043 Extraction unit. Tecator, Foss NA 7682 Executive Drive, Eden Prairie, MN 55344
- NDF: Modified procedure of Van Soest et al. (1991) with whatman 934-AH glass micro-fiber filters with 1.5um particle retention
- Starch: Hall, 2009
- Sugar: Dubois et al. 1956

4.5.4. Determination of protein and carbohydrate sub-fractions (CNCPS 6.5)

CNCPS version 6.5 was used to determine protein and carbohydrate sub-fractions, estimated parameters are listed below:

PA1 fraction (ammonia) with a degradation rate (Kd) of 200 %/h

PA2 fraction (soluble true protein). Kd= 27 %/h

PB1 fraction (insoluble true protein). Kd= 12 %/h

PB2 fraction (fiber-bound protein). Kd= 5 %/h

PC fraction (indigestible protein).

CA1 fraction (volatile fatty acids). Kd= 0 %/h

CA2 fraction (lactic acid). Kd= 7 %/h

CA3 fraction. Kd= 5 %/h

CA4 fraction (water soluble carbohydrates). Kd= 40 %/h

CB1 fraction (starch). Kd= 25 %/h

CB2 fraction (soluble fiber). Kd= 30 %/h

CB3 fraction (digestible fiber). Kd= 5 %/h

CC fraction (indigestible fiber).

All equations described in Chapter 3 (3.5.3) were used in the current chapter for protein and CHO estimation.

4.5.5. Determination of energy values

NRC dairy (2001) was used to determine the energy values of the processed samples using the data from the chemical analyses. Estimated parameters included: truly digestible CP (tdCP), truly digestible non-fiber carbohydrates (tdNFC), truly digestible NDF (tdNDF), and truly digestible fatty acids (tdFA), total digestible nutrients at 1x maintenance (TDN_{1x}) digestible energy at maintenance level (DE_{1X}), digestible energy of production at 3x maintenance level (DE_{p3x}), metabolizable energy of production at 3x maintenance level (ME_{p3x}), and net energy of production at 3x maintenance level (NE_{Lp3x}). To determine metabolizable energy (ME), net energy for maintenance (NE_m), and net energy for gain (NE_g) NRC beef (1996) was used.

4.5.6. Rumen in situ incubation procedure and rumen degradation kinetics of feed nutrients degradation kinetics

Four cannulated Holstein Friesian milking cows were used for the *in situ* study. The cows were kept in tie stalls during the period of sampling and they were milked two times a day in a milking parlor. Cows were fed a total mixed ration (TMR) two times daily based on barley silage, alfalfa hay, energizer, pellets and rolled barley; the average daily intake was 28 kg of DM. This study was performed in the Rayner Dairy Research and Teaching Facility, Saskatoon, SK, Canada. Information related to the cows is presented in table 4.1. Previously rolled samples were weighed (7.5 g) into 10 x 20 cm nylon bags with a size pore of 41 μm.

Incubation times in the rumen were completed at 0, 2, 4, 8, 12, and 24 h following first-order degradation kinetics described by Ørskov and McDonald (1979) and Tamminga et al. (1994). Degradation parameters and fractions were calculated as described by Tamminga et al. (1994), Heendeniya et al. (2012), and Damiran et al. (2013). Detailed description of the formulas used for determination of rumen degradation kinetics were given in Chapter 3 (3.5.5.).

Table 4.1. Characteristics of milking cows used in the *in situ* study.

# of animal	Stock #	Age	DIM	Average production
1	906	6 years	360	33 kg
2	950	5 years	452	31 kg
3	874	6 years	399	26 kg
4	853	7 years	277	37 kg

Notes: DIM: days in milk. Average production per day

4.5.7. *Intestinal digestibility of feed nutrients using a three-step in vitro technique*

Intestinal digestibility of feed nutrients was estimated by following the procedure described by Calsamiglia and Stern (1995) using 12 h residue samples from rumen incubation.

4.5.8. Hourly effective rumen degradation ratios and potential nitrogen to energy synchronization in the rumen

Effective degradation parameters were computed following the equations described in Sinclair et al. (1993) and Tamminga et al. (1990). Detailed information was included in Chapter 3 (3.5.7).

4.5.9. Prediction of truly digestible nutrient supply to the small intestine in dairy cattle

The prediction of true nutrient supply to the small intestine was determined using the DVE/OEB Dutch system described in Tamminga et al. (1994) and the NRC dairy model obtained from NRC (2001). Parameters such as rumen undegraded feed protein, microbial protein synthesis, endogenous protein loss, truly digested and absorbed protein in the small intestine, and the degraded protein balance were included in this analysis. Detailed explanation of equations used in these models were described in Chapter 3 (3.6.7.).

4.5.10. Statistical analyses

Results from chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios, intestinal digestibility of protein, predicted truly absorbed protein supply and feed milk values were analyzed using the Mixed model procedure of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US). CRD was used as experimental design with a 2 x 3 factorial treatment arrangement with the first factor related to tannin level (low and normal) and the second factor including the treatments (raw, Steam Pressure, and Microwave Irradiation). The model used for the analysis was as follows:

$$Y_{ij} = \mu + F_i + H_j + (F_i \times H_j) + e_{ij},$$

Where Y_{ij} was the observation of the dependent variable ij, μ was the population mean for the variable, F_i the effect of tannin level (i= 1,2); H_j the effect of heat treatments (j= 1,2,3), $F_i \times H_j$ the interaction between variables, and e_{ij} the random error associated with observation ij.

PROC NLIN-Gauss-Newton method of SAS was used to fit the rumen degradation data to the model. The difference among treatments was evaluated with a multiple comparison analysis using the Tukey method.

For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

4.6. Results and Discussion

4.6.1. Effect of heat processing on the nutrient profiles of faba bean seeds with low and normal tannin level

Table 4.2. shows the complete nutrient profiles of raw and heat treated faba bean. Dry matter (DM) was increased ($P < 0.05$) by both Steam Pressure (SP) (+ 2.0 %) and Microwave Irradiation (MI) (+ 4.8 %) treatments of which MI had a greater effect than SP. Ether extract was also increased ($P < 0.05$) with SP compared to MI but remained the same as the Control treatment (raw seeds). The

soluble crude protein (SCP) was higher ($P < 0.05$) in the low tannin types (LT) (+ 1.6 % DM) compared to normal tannin types (NT) and SP decreased ($P < 0.05$) the SCP content while MI showed no difference from the Control. LT presented a lower acid detergent insoluble crude protein (ADICP) (- 0.1 % DM) than NT and both heat treatments increased ADICP content (SP: + 0.4, MI: + 0.4 % DM). Effects related to carbohydrates profile showed a higher percentage of starch and non-structural carbohydrates (NSC) (+ 2.1 % and 2.2 % DM, respectively. $P < 0.05$) in LT compared to NT. SP and MI increased ($P < 0.05$) the starch level (+ 4.3 and + 3.7 % DM, respectively) compared to the Control but were similar between each other. Sugar content was affected by heat processing as well, decreasing the sugar level by 2.5 % DM in both treatments compared to the Control ($P < 0.05$). Acid detergent fiber (ADF) was increased ($P < 0.05$) with MI (+ 2.6 % DM) while SP remain the same as the Control ($P > 0.10$). Acid detergent lignin (ADL) was lower in LT (- 0.3 % DM), SP had a greater effect ($P < 0.05$) on ADL content compared to MI (- 1.9 % NDF) and the Control (- 3.8 % NDF). Cellulose was only increased ($P < 0.05$) by MI (+ 2.3 %) and remained the same with SP compared to the Control treatment. Organic matter (OM), neutral detergent fiber (NDF), and hemicellulose showed no significant effects ($P > 0.10$) of heat processing methods on different tannin types of faba bean. Mustafa et al. (1998) found a decrease in SCP, increase of NDICP, and no changes in ADICP after autoclaving field peas for 30 min at 127 °C. These authors stated that moderate heat processing aims to reduce SCP, increase NDICP with little increase in ADICP as this last parameter is the indicator of unavailable protein content in the feed.

Table 4.2. Chemical profile of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Basic chemical profile										
DM (%)	94.5	95.0	0.23	92.5^{c}	94.5^{b}	97.2^{a}	0.29	0.17	<0.01	0.51
Ash (% DM)	3.2	3.1	0.16	3.3	3.0	3.1	0.19	0.60	0.48	0.28
EE (% DM)	1.2	1.3	0.07	0.9^{b}	1.8^{a}	1.0^{b}	0.08	0.78	<0.01	0.23
FA (% DM)	0.3	0.4	0.05	0.0^{b}	0.8^{a}	0.1^{b}	0.07	0.23	<0.01	0.31
OM (% DM)	96.8	97.0	0.16	96.7	97.0	96.9	0.19	0.60	0.48	0.28
Protein profile										
CP (% DM)	27.7	28.3	0.28	27.9^{ab}	28.8^{a}	27.3^{b}	0.34	0.11	0.03	0.80
SCP (% DM)	14.8^{a}	13.2^{b}	0.29	18.9^{a}	4.5^{b}	18.6^{a}	0.35	<0.01	<0.01	0.09
SCP (% CP)	54.1	47.1	0.77	67.9	15.7	68.1	0.94	<0.01	<0.01	0.01
NDICP (% DM)	2.2	1.5	0.31	1.9	1.6	2.1	0.38	0.10	0.69	0.08
NDICP (% CP)	8.8	5.2	1.13	6.6	5.7	7.8	1.39	0.09	0.59	0.08
ADICP (% DM)	0.2^{b}	0.4^{a}	0.03	0.0^{b}	0.4^{a}	0.4^{a}	0.03	<0.01	<0.01	0.05
ADICP (% CP)	0.8^{b}	1.3^{a}	0.09	0.1^{b}	1.5^{a}	1.6^{a}	0.11	<0.01	<0.01	0.05
Carbohydrate profile										
CHO (% DM)	67.9	67.4	0.28	67.9^{a}	66.5^{b}	68.6^{a}	0.35	0.18	<0.01	0.95
Starch (% DM)	40.0^{a}	38.0^{b}	0.53	36.3^{b}	40.7^{a}	40.0^{a}	0.64	0.02	<0.01	0.76
Starch (% NFC)	77.3	77.0	1.79	69.2^{b}	83.8^{a}	78.4^{a}	2.19	0.92	<0.01	0.47
Sugar (% DM)	2.7	2.5	0.13	4.2^{a}	1.8^{b}	1.8^{b}	0.16	0.41	<0.01	0.51
Sugar (% NFC)	5.1	4.9	0.25	8.0^{a}	3.8^{b}	3.4^{b}	0.30	0.65	<0.01	0.53
NFC (% DM)	51.9	49.6	0.93	52.4	48.6	51.2	1.14	0.10	0.09	0.67
NFC (% CHO)	76.4	73.6	1.38	77.3	73.2	74.6	1.64	0.16	0.24	0.61
NSC (% DM)	42.7^{a}	40.5^{b}	0.60	40.5	42.4	41.7	0.73	0.02	0.23	0.92

Table 4.2. *Cont'd*. Chemical profile of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Fiber profile										
NDF (% DM)	18.2	19.3	0.86	17.3	19.5	19.5	1.06	0.43	0.27	0.60
ADF (% DM)	11.6	12.0	0.29	10.8^{b}	11.2^{b}	13.4^{a}	0.35	0.32	<0.01	0.35
ADF (% NDF)	63.6	63.4	2.46	62.4	58.7	69.6	3.02	0.95	0.07	0.19
ADL (% DM)	0.6^{b}	0.9^{a}	0.03	0.3^{c}	1.1^{a}	0.7^{b}	0.05	<0.01	<0.01	0.74
ADL (% NDF)	3.1	4.5	0.32	1.9^{c}	5.7^{a}	3.8^{b}	0.40	0.01	<0.01	0.68
NDFn (% DM)	16.0	17.8	0.90	15.4	17.9	17.4	1.11	0.19	0.30	0.61
Hemicellulose (% DM)	6.7	7.3	0.78	6.5	8.3	6.1	0.96	0.61	0.26	0.28
Cellulose (% DM)	11.0	11.1	0.28	10.4^{b}	10.1^{b}	12.9^{a}	0.35	0.74	<0.01	0.37

Notes: $^{a-b}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparison using Tukey method. DM: dry matter; EE: ether extract (crude fat); CP: crude protein; OM: organic matter; FA: fatty acids; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-soluble carbohydrate. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

4.6.2. Effect of heat processing on protein and carbohydrate sub-fractions of faba bean seeds with low and normal tannin level

Results from protein and carbohydrate sub-fractions based on CNCPS 6.5. are presented in Table 4.3. The rapidly degradable protein fraction (PA2) showed interactions between heat treatment and tannin level (low and normal), the Control treatment in LT was higher (+ 12.9 % CP) than NT; SP sharply reduced ($P < 0.05$) the PA2 level in LT and NT (- 57.5, - 46.9 %, respectively). On the other hand, MI treatment was not different from the Control treatment in LT and NT, in fact, it caused a reduction of 3.3 % and 3.6 % in PA2 content, respectively. Levels of moderately degradable protein fraction (PB1) and indigestible protein fraction (PC) were higher ($P < 0.05$) in NT (+ 9.9 % CP and + 0.5 % CP) compared to LT. SP significantly increased ($P < 0.05$) the PB1 value (+ 53.1 % CP) while MI remained the same as the Control. PC content was higher than the Control in both thermal treatments (SP and MI). These results agree with data published by Mustafa et al. (1998), where the rapidly degradable fraction was decreased and the intermediately and slowly degradable fractions were increased after autoclaving.

In terms of CHO sub-fractions, SP decreased ($P < 0.05$) the values of the rapidly degradable carbohydrate fraction (CA4) (- 3.6 % CHO), intermediately degradable carbohydrate fraction (CB2) (- 8.2 % CHO), and the unavailable neutral detergent fiber (CC) (- 1.4 % CHO); MI decreased ($P < 0.05$) CA4 and CC with a similar effect to SP. CB1 was greater ($P < 0.05$) with SP and MI but were similar among both treatments.

Table 4.3. Protein and carbohydrate sub-fractions, degradable and bypass fractions of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Protein sub-fractions										
PA2 (% CP)	54.1	47.1	0.77	67.9	15.7	68.1	0.94	<0.01	<0.01	0.01
PB1 (% CP)	37.8^{b}	47.7^{a}	1.16	25.5^{b}	78.6^{a}	24.2^{b}	1.42	<0.01	<0.01	0.12
PB2 (% CP)	7.3	3.9	1.12	6.5	4.2	6.2	1.37	0.05	0.45	0.06
PC (% CP)	0.8^{b}	1.3^{a}	0.09	0.1^{b}	1.5^{a}	1.6^{a}	0.11	<0.01	<0.01	0.054
PA2 (% tp)	54.5	47.6	0.78	68.0	16.0	69.1	0.95	<0.01	<0.01	0.01
PB1 (% tp)	38.2^{b}	48.5^{a}	1.18	25.5^{b}	79.8^{a}	24.6^{b}	1.44	<0.01	<0.01	0.13
PB2 (% tp)	7.4	3.9	1.14	6.5	4.2	6.3	1.39	0.05	0.47	0.06
Carbohydrate sub- fractions										
CA4 (% CHO)	4.0	3.8	0.19	6.2^{a}	2.6^{b}	2.6^{b}	0.24	0.47	<0.01	0.46
CB1 (% CHO)	58.9	56.4	0.87	53.5^{b}	61.2^{a}	58.3^{a}	1.07	0.06	<0.01	0.83
CB2 (% CHO)	13.6	13.5	1.54	17.6^{a}	9.4^{b}	13.8^{ab}	1.88	0.97	0.03	0.58
CB3 (% CHO)	22.9	25.5	1.36	21.0	26.5	25.0	1.67	0.21	0.09	0.57
CC (% CHO)	0.6	0.9	0.28	1.7^{a}	0.3^{b}	0.3^{b}	0.34	0.46	0.02	0.85

Note: $^{a-b}$ Means with different letters in the same row are significantly different (P<0.05). PA2 = rapidly degradable protein fraction; PB1 = moderately degradable protein fraction; PB2 = slowly degradable protein fraction; PC = indigestible or unavailable protein fraction; tp = true protein; CA4 = rapidly degradable carbohydrate fraction (sugar); CB1 = intermediately degradable carbohydrate fraction (starch); CB2 = intermediately degradable carbohydrate fraction (soluble fiber); CB3 = slowly degradable carbohydrate fraction (digestible fiber); CC = unavailable neutral detergent fiber (indigestible fiber; SEM, Standard Error of Mean. Multi-treatment comparison using Tukey method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

4.6.3. Effect of heat processing on energy values of faba bean seeds with low and normal tannin level

Results from truly digestible parameters of non-fiber carbohydrates (NFC), fatty acids (FA), and neutral detergent fiber (NDF) were not significantly different ($P < 0.05$) among treatments or tannin level. Detailed data of energy values from raw and heat treated faba bean based on NRC dairy 2001 and NRC beef 1996 are presented in Table 4.4. The truly digestible crude protein (tdCP) content was affected by both heat treatments. SP slightly increased tdCP (+ 0.7 % DM) while MI slightly decreased this value (- 0.8 % DM); however, SP and MI were not statistically different from the Control. The total digestible energy (TDN) was higher ($P < 0.05$) in LT (+ 1.1 % DM) than NT; MI did not change TDN level, but SP decreased the amount by 3.0 % DM related to the Control treatment. All energy values were reduced ($P < 0.05$) with both thermal treatments: digestible energy (DE_{1x}), digestible energy at a production level of 3 times maintenance (DE_{p3x}), metabolizable energy dairy (ME_{dairy}), net energy at production level of 3 times maintenance (NE_{Lp3x}), metabolizable energy beef (ME_{beef}), net energy for maintenance (NE_m), and net energy for gain (NE_g). No difference ($P > 0.10$) was observed between SP and MI in the previously mentioned energy values.

Table 4.4. Energy values of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Truly Digestible Nutrients										
tdNFC (% DM)	52.9	50.6	0.95	53.4	49.6	52.3	1.16	0.10	0.09	0.66
tdCP (% DM)	27.6	28.2	0.28	27.9^{ab}	28.6^{a}	27.1^{b}	0.34	0.14	0.03	0.75
tdFA (% DM)	0.0	0.0	0.01	0.0	0.0	0.0	0.01	0.33	0.28	0.70
tdNDF (% DM)	10.4	11.0	0.68	10.5	10.7	11.0	0.83	0.53	0.89	0.61
Total Digestible Nutrients										
TDN_{1x} (% DM)	83.9^{a}	82.8^{b}	0.33	84.9^{a}	81.8^{b}	83.4^{a}	0.40	0.03	<0.01	0.46
Energy values										
DE_{1X} (Mcal/kg)	3.90	3.87	0.016	3.95^{a}	3.83^{b}	3.88^{b}	0.019	0.12	<0.01	0.45
DEp_{3X} (Mcal/kg)	3.58	3.55	0.014	3.63^{a}	3.52^{b}	3.56^{b}	0.018	0.12	<0.01	0.48
ME (Mcal/kg) - dairy	3.17	3.14	0.014	3.21^{a}	3.10^{b}	3.15^{b}	0.017	0.09	<0.01	0.43
NEL_{3X} (Mcal/kg)	2.04	2.01	0.009	2.07^{a}	1.99^{b}	2.02^{b}	0.012	0.09	<0.01	0.34
ME (Mcal/kg) - beef	3.20	3.17	0.013	3.24^{a}	3.14^{b}	3.18^{b}	0.016	0.09	<0.01	0.42
NEm (Mcal/kg)	2.20	2.17	0.011	2.23^{a}	2.15^{b}	2.18^{b}	0.013	0.12	<0.01	0.50
NEg (Mcal/kg)	1.51	1.49	0.009	1.54^{a}	1.47^{b}	1.50^{b}	0.011	0.12	<0.01	0.49

Notes: $^{a-b}$ Means with different letters in the same row are significantly different (P<0.05). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Values based on NRC-2001 Dairy and NRC-1996 Beef. tdNFC, truly digestible non-fiber carbohydrate; tdCP: truly digestible crude protein; tdFA: truly digestible fatty acid; tdNDF: truly digestible neutral detergent fiber. TDN1×: total digestible nutrient at one time maintenance. DE1×: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; NE_L3×: net energy for lactation at production level of intake (3×); NEm: net energy for maintenance; NEg: net energy for growth. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

4.6.4. Effect of heat processing on in situ rumen degradation of dry matter of faba bean seeds with low and normal tannin level

Dry matter (DM) degradation kinetics of raw and heat treated faba bean are shown in Table 4.5. The rate of degradation (Kd) of DM was increased ($P < 0.05$) only by MI (+ 6.60 %/h), SP was similar to the Control treatment (raw seeds). The soluble fraction (S) in the rumen was 3.1 % higher in LT than the NT; a greater reduction ($P < 0.05$) of S was observed with SP (- 19.0 %) and MI decreased ($P < 0.05$) S by 7.4 %. The degradable (D) and undegradable (U) fractions were affected by the interaction between tannin level and heat treatment: in LT and NT SP increased ($P < 0.05$) the D fraction by 23.6 and 17.3 %, respectively while MI did not differ from the Control. In the U fraction, the effect of heat was only observed with MI in NT with an increase of 10.7 %. Rumen bypass or undegraded dry matter (BDM) was higher ($P < 0.05$) in NT (+ 4.3 %) compared to LT and was increased ($P < 0.05$) with SP (+ 16.3 %) compared to the Control treatment. The effective degradability of DM (EDDM) was not different ($P > 0.10$) between Control and MI and was lower ($P < 0.05$) with SP.

4.6.5. Effect of heat processing on in situ rumen degradation of crude protein and starch of faba bean seeds with low and normal tannin level

Results from degradation parameters of crude protein (CP) are presented in Table 4.6. The degradation rate (Kd) was decreased ($P < 0.05$) with SP (- 4.14 %/h) and increased ($P < 0.05$) with MI (+ 7.87 %/h). The soluble (S) fraction was decreased ($P < 0.05$) by both heat treatments SP (- 27.8 %) and MI (- 7.8 %) while these treatments increased ($P < 0.05$) the degradable (D) fraction SP (+ 27.8 %) and MI (+ 7.0 %). Interaction between tannin level and heat treatment was observed in the undegradable (U) protein fraction of NT where MI increased ($P < 0.05$) this parameter. These results were similar to those obtained in field peas by Mustafa et al. (1998). The effects of

SP and MI also agreed with previous published data by Yu (2005b) which observed that pressure toasting decreased the S fraction and increased D fraction. In the same study the Kd value was decreased as well but this disagrees with the results of the present MI effect. Goelema et al. (1998) reported an increased in Kd with a consequent decrease of RUP when using pelleting in a mixture of broken peas, lupins and faba bean. Goelema et al. (1998) found that pressure toasting at 132 °C for 3 min increased RUP content by decreasing the S fraction and Kd which is mainly related with the Millard's reactions and crosslinking among and between proteins as a consequence of heat. Results reported by Yu et al. (1998) about dry roasted faba bean, agreed with the effects of MI protein degradation features of this study (S, D, Kd and BCP).

The content of rumen undegradable protein based on DVE system (BCP) and NRC model (RUP) in percentage and g/kg DM units were changed as well with both heat treatments, NT had greater ($P < 0.05$) values of BCP and RUP compared to LT. SP increased ($P < 0.05$) BCP amount by 71 g/kg and RUP by 64 g/kg compared to the Control. In contrast, MI decreased ($P < 0.05$) these values by 16 g/kg and 14 g/kg, respectively. On the other hand, the percentage of effective degraded crude protein (EDCP) was reduced ($P < 0.05$) with SP (- 21.6 %) and increased ($P < 0.05$) with MI (+ 4.6 %). In EDCP (g/ kg DM), SP also decreased ($P < 0.05$) the value by 56 g/kg while MI remain the same as the Control treatment.

Starch degradation kinetics are presented in Table 4.7. The rate of degradation (Kd) decreased ($P < 0.05$) with SP (- 7.42 %/h) and increased ($P < 0.05$) with MI (+ 5.19 %/h) compared to the Control treatment. The soluble (S) fraction was reduced ($P < 0.05$) with SP (-28.1 %) and MI (-17.1 %), increasing the degradable (D) fraction in the rumen. Bypass starch (BST) was affected ($P < 0.05$) only by SP, increasing its value compared to the Control (+ 130 g/kg). Goelema et al. (1998) reported decreased S, increased Kd and higher BST when pressure toasting broken faba

bean. The effective degraded starch (EDST) content was higher ($P < 0.05$) in LT (+ 25 g/kg) compared to NT; a greater effect on EDST was observed with MI (+ 31 g/kg) compared to the Control. The main objective of heat processing treatments is to decrease the highly and easily soluble and rapidly degradable protein and CHO fractions in the rumen resulting in increased available amino acids and starch supply to the small intestine. In this study, Steam Pressure had a greater impact on the rumen degradation kinetics of CP and starch of faba bean grown in western Canada than Microwaving.

4.6.6. Effect of heat processing on in situ rumen degradation of neutral detergent fiber of faba bean seeds with low and normal tannin level

Table 4.8. shows detailed data related to the degradation parameters of neutral detergent fiber (NDF). NT showed a greater ($P < 0.05$) level of washable (S) fraction than LT. Both SP and MI increased S (+ 14.7 % and + 16.8 %, respectively, $P < 0.05$). The undegradable (U) fraction was 12.2 % lower ($P < 0.05$) in LT compared to NT. The undigestible neutral detergent fiber ($uNDF_{288h}$) was reduced ($P < 0.05$) by both SP and MI by 0.9 % compared to the Control; however, they were similar between each other. Degradation rate (Kd), rumen undegradable NDF (RUNDF), and the effective degradable NDF (EDNDF) were not significant different ($P > 0.10$) between LT and NT and among different heat processing treatments.

Table 4.5. Degradation kinetics of dry matter (DM) in raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Dry matter degradation										
Kd (%/h)	10.08	9.62	0.716	8.71^{b}	5.55^{b}	15.31^{a}	0.887	0.66	<0.01	0.46
T0 (h)	1.8	1.6	0.19	2.9^{a}	0.9^{b}	1.4^{b}	0.23	0.36	<0.01	0.87
Residue (0 h, %)	74.7^{b}	77.8^{a}	0.84	67.4^{c}	86.4^{a}	74.9^{b}	1.03	0.02	<0.01	0.55
S (%)	25.3^{a}	22.2^{b}	0.84	32.6^{a}	13.6^{c}	25.2^{b}	1.03	0.02	<0.01	0.55
D (%)	72.8	74.2	1.25	66.0^{b}	86.4^{a}	68.1^{b}	1.53	0.44	<0.01	0.04
U (%)	1.9	3.6	0.82	1.5	0.0	6.8	1.01	0.18	<0.01	0.01
% BDM = % RUDM	31.4^{b}	35.6^{a}	0.87	28.9^{b}	45.2^{a}	26.3^{b}	1.07	<0.01	<0.01	0.63
% EDDM	68.6^{a}	64.4^{b}	0.87	71.1^{a}	54.8^{b}	73.7^{a}	1.07	<0.01	<0.01	0.63

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.6. Degradation kinetics of crude protein (CP) in raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Crude protein degradation										
Kd (%/h)	11.44	11.43	0.636	10.19^{b}	6.05^{c}	18.06^{a}	0.779	0.99	<0.01	0.53
T0 (h)	2.5	3.5	0.17	2.9	3.1	2.9	0.20	<0.01	0.74	0.01
Residue (0 h, %)	69.5	73.0	1.20	59.4^{c}	87.8^{a}	67.1^{b}	1.47	0.06	<0.01	0.19
S (%)	30.5	27.0	1.20	40.6^{a}	12.8^{c}	32.9^{b}	1.47	0.06	<0.01	0.19
D (%)	69.5	72.5	1.16	59.4^{c}	87.2^{a}	66.4^{b}	1.42	0.10	<0.01	0.31
U (%)	0.0	0.5	0.13	0.0	0.0	0.8	0.16	0.01	0.01	0.01
% BCP = % RUP	26.6	28.9	0.91	22.1^{b}	43.7^{a}	17.5^{c}	1.11	0.09	<0.01	0.27
BCP (g/kg DM, DVE)	82^{b}	92^{a}	3.0	69^{b}	140^{a}	53^{c}	3.7	0.04	<0.01	0.27
RUP (g/kg DM, NRC)	74^{b}	83^{a}	2.7	62^{b}	126^{a}	48^{c}	3.3	0.04	<0.01	0.27
% EDCP = % RDP	73.4	71.0	0.90	77.9^{b}	56.3^{c}	82.5^{a}	1.11	0.09	<0.01	0.27
EDCP (g/kg DM)	202	201	3.3	218^{a}	162^{b}	225^{a}	4.0	0.68	<0.01	0.24

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Kd: rate of degradation of D fraction (%/h); U: rumen undegradable fraction; D: potentially degradable fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.7. Degradation kinetics of starch (ST) in raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Starch degradation										
Kd (%/h)	13.47	12.64	0.925	13.80^{b}	6.38^{c}	18.99^{a}	1.132	0.54	<0.01	0.19
T0 (h)	3.1	3.1	0.38	5.5^{a}	1.6^{b}	2.2^{b}	0.47	0.92	<0.01	0.20
Residue (0 h, %)	83.9	88.3	1.61	70.7^{c}	99.8^{a}	87.8^{b}	1.97	0.08	<0.01	0.30
S (%)	16.1	11.7	1.61	29.3^{a}	0.3^{c}	12.2^{b}	1.97	0.08	<0.01	0.30
D (%)	83.9	88.3	1.61	70.7^{c}	99.8^{a}	87.8^{b}	1.97	0.08	<0.01	0.30
% BSt	29.4	31.8	1.03	21.9^{b}	48.5^{a}	21.4^{b}	1.26	0.13	<0.01	0.30
BSt (g/kg DM)	132	136	5.2	89^{b}	219^{a}	95^{b}	6.4	0.53	<0.01	0.38
% EDSt	70.6	68.2	1.03	78.1^{a}	51.5^{b}	78.6^{a}	1.26	0.13	<0.01	0.30
EDST (g/kg DM)	282^{a}	257^{b}	4.8	284^{b}	209^{c}	315^{a}	5.8	<0.01	<0.01	0.34

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.8. Degradation kinetics of neutral detergent fiber (NDF) and indigestible detergent fiber ($uNDF_{288h}$) in raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Fiber degradation										
Kd (%/h)	11.32	18.62	2.730	11.6	19.66	13.60	3.343	0.08	0.25	0.36
T0 (h)	1.0	1.4	0.35	0.6	1.9	1.02	0.43	0.44	0.14	0.10
Residue (0h, %)	93.4[a]	84.6[b]	2.78	99.5[a]	84.8[b]	82.7[b]	3.40	0.04	0.01	0.13
S (%)	6.6[b]	15.4[a]	2.78	0.5[b]	15.2[a]	17.3[a]	3.40	0.04	0.01	0.13
D (%)	63.8[a]	42.9[b]	4.61	64.7	45.1	50.2	5.65	0.01	0.08	0.19
U (%)	29.6[b]	41.7[a]	3.77	34.8	39.7	32.5	4.61	0.04	0.55	0.23
% BNDF = % RUNDF	53.9	55.9	2.29	58.8	52.8	53.2	2.80	0.54	0.28	0.36
RUNDF (g/kg DM, NRC)	98	106	3.1	102	101	103	3.9	0.09	0.92	0.52
% EDNDF = % RDNDF	46.1	44.1	2.29	41.2	47.2	46.8	2.80	0.54	0.28	0.36
EDNDF = RDNDF (g/kg DM)	85	87	8.2	71	94	92	10.0	0.88	0.24	0.36
$uNDF_{288h}$ (% DM)	0.41	0.60	0.176	1.08[a]	0.21[b]	0.22[b]	0.216	0.48	0.02	0.87

Notes: [a-b] Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. Kd: the degradation rate of D fraction; T0: lag time; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. uNDF: indigestible neutral detergent fiber at 288 h based on CNCPS 6.5). Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

4.6.7. Effect of heat processing on intestinal digestibility of feed nutrients of faba bean seeds with low and normal tannin level

Intestinal digestibility of DM and CP is presented in Table 4.9. Heat treatments did not affect the intestinal digestibility of rumen undegraded or bypass dry matter (dBDM) or the content of total digested crude protein in g per kg of DM ($P > 0.10$). Changes were observed in the digestibility of rumen undegraded crude protein (dIDP). LT showed a higher level of dIDP (+ 9 %), MI had a greater decrease (- 11.1 %) compared to SP but both treatments were not different from the Control. The intestinal digestible rumen bypass dry matter (IDBDM) was greater with SP (+ 9.2 %) while MI was not different compared to the Control. The intestinal absorbable digested rumen bypass protein (IADP) content (g/kg DM) and percentage were affected by both SP and MI; a significant increase ($P < 0.05$) was observed with SP (+ 63 g/kg, + 21.2 %) while MI decrease the IADP value by 13 g/kg and 4.3 % compared to the Control treatment. In terms of total digested DM (TDDM) in the digestive tract, LT had higher content than NT (+ 5.2 %) and this was decreased with SP (- 7 %) compared to the Control. The parameters of intestinal digestibility of starch are presented in Table 4.10. The digestibility of bypass starch (dBST) was higher ($P < 0.05$) with MI (+ 19.1 %) compared to SP; however, both treatments were not different from the Control. There was an interaction between tannin level and heat treatment for the intestinal digested bypass starch (IDBST) ($P < 0.05$). The levels of IDBST were similar in both LT and NT; however, SP increased ($P < 0.05$) the IDBST by 24.4 % and 119 g/kg in LT and by 14.9 % and 76 g/kg in NT compared to the Control; IDBST content with MI was not different from the one in the raw seeds. The total digested starch (TDST) in the digestive tract remained the same between the Control and MI but SP reduced the value by 6.9 %. The content of TDST in g per kg was higher in LT than NT and was increased with MI (+ 50 g/kg) while SP was not different from the Control or MI. Data of

intestinal digestibility of NDF showed differences between tannin level and heat treatment ($P < 0.05$), detailed results are represented in Table 4.11.

4.6.8. Effect of heat processing on hourly effective rumen degradation ratios and potential nitrogen to energy synchronization in the rumen of faba bean seeds with low and normal tannin level

Data of potentially available N to available CHO is presented in Table 4.12. A difference ($P < 0.05$) was observed in the potentially available effective degraded N to effective degraded CHO (ED_N/ED_CHO), ($P < 0.05$) MI reduced the ratio from 84 g/kg to 74 g/kg while SP showed no difference from the Control and MI. In the first 8 h of degradation (short incubation period), no significant difference ($P > 0.10$) was observed among treatments, but from 12 h to 24 h (longer incubations) values begin to increase in SP and decreased with MI. Results are represented in Figures 4.1 and 4.2.

Table 4.9. Intestinal digestibility and total tract digestion (DM and CP) of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Dry matter digestion										
% dBDM	64.8	56.4	2.82	60.0	59.8	61.9	3.45	0.06	0.90	0.12
% IDBDM	20.5	19.6	0.93	17.4^{b}	26.6^{a}	16.2^{b}	1.14	0.50	<0.01	0.09
% TDDM	89.1^{a}	83.9^{b}	1.41	88.4^{a}	81.4^{b}	89.9^{a}	1.72	0.02	0.01	0.14
Crude protein digestion										
% dIDP	87.1^{a}	78.1^{b}	2.27	80.2^{ab}	89.3^{a}	78.2^{b}	2.77	0.02	0.03	0.66
IADP (g/kg DM)	65.4	66.9	2.52	49.5^{b}	112.2^{a}	36.9^{c}	3.09	0.67	<0.01	0.27
IADP (g/kg CP)	235	234	9.1	178^{b}	390^{a}	135^{c}	11.1	0.97	<0.01	0.33
TDP (g/kg DM)	268	268	2.4	267	274	262	2.9	0.91	0.05	0.72
TDP (g/kg CP)	969^{a}	944^{b}	4.9	956	953	961	6.1	<0.01	0.68	0.97
%IADP (% CP)	23.5	23.4	0.91	17.8^{b}	39.0^{a}	13.5^{c}	1.11	0.97	<0.01	0.33
%TDP (% CP)	96.9^{a}	94.4^{b}	0.49	95.6	95.3	96.1	0.61	<0.01	0.68	0.97

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IADP: intestinal digestible crude protein; TDP: total digested crude protein. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.10. Intestinal digestibility and total tract digestion (starch) of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Starch digestion										
% dBST	86.2	86.8	4.03	83.0^{ab}	78.7^{b}	97.8^{a}	4.938	0.91	0.04	0.09
% IDBST	25.5	25.9	1.28	18.2	37.9	20.9	1.57	0.81	<0.01	0.04
IDBST (g/kg DM)	114	110	5.2	73	171	93	6.4	0.61	<0.01	0.02
% TDST	96.1	94.1	1.38	96.3^{a}	89.4^{b}	99.6^{a}	1.70	0.34	<0.01	0.07
TDST (g/kg DM)	396^{a}	367^{b}	6.0	357^{b}	380^{ab}	407^{a}	7.4	0.01	<0.01	0.17

Notes: $^{a-b}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.11. Intestinal digestibility and total tract digestion (NDF) of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Low tannin genotypes			Normal tannin genotypes			SEM	P value		
	Control (n= 3)	Steam Pressure (n= 3)	Microwave Irradiation (n= 3)	Control (n= 3)	Steam Pressure (n= 3)	Microwave Irradiation (n= 3)		Tannin level (T)	Heat treatment (Heat)	T x Heat
Fiber digestion										
% dBNDF	20.6^{abc}	27.1^{ab}	33.8^{a}	20.7^{abc}	8.7^{c}	14.1^{bc}	3.26	<0.01	0.22	0.02
% IDBNDF	11.7^{ab}	14.8^{a}	16.9^{a}	12.6^{ab}	4.0^{b}	8.0^{ab}	2.01	<0.01	0.28	0.03
IDBNDF (g/kg DM)	20^{abc}	27^{ab}	33^{a}	23^{abc}	9^{c}	15^{bc}	3.3	<0.01	0.20	0.01
% TDNDF	54.9	59.8	67.1	51.8	53.6	51.5	4.02	0.03	0.37	0.31
TDNDF (g/kg DM)	93	107	133	92	116	99	14.8	0.47	0.27	0.38

Notes: $^{a-c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.12. Potentially available N to available CHO synchronization of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
N/CHO (g/kg)	76	80	1.6	84^{a}	77^{ab}	74^{b}	1.9	0.14	0.01	0.56
ED_N/ED_CHO (g/kg)	84^{b}	92^{a}	2.4	93	83	88	2.9	0.05	0.07	0.08
Hourly effective degradation ratios at individual times (g/kg)										
h0	154	176	29.6	171	136	189	36.2	0.63	0.63	0.92
h2	61	74	6.1	59	63	80	7.5	0.16	0.17	0.21
h4	62	69	2.2	62	67	67	2.7	0.06	0.32	0.45
h8	63	67	4.8	67	74	56	5.8	0.55	0.14	0.55
h12	63	69	5.8	71^{ab}	78^{a}	49^{b}	7.1	0.44	0.03	0.65
h16	61	71	6.6	74^{a}	82^{a}	41^{b}	8.1	0.30	0.01	0.84
h20	59	72	7.8	78^{a}	84^{a}	34^{b}	9.6	0.26	0.01	0.87
h24	58	73	9.4	81^{a}	87^{a}	28^{b}	11.5	0.27	0.01	0.79

Notes: $^{a-b}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. ED: effective degradability; CHO: carbohydrates. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

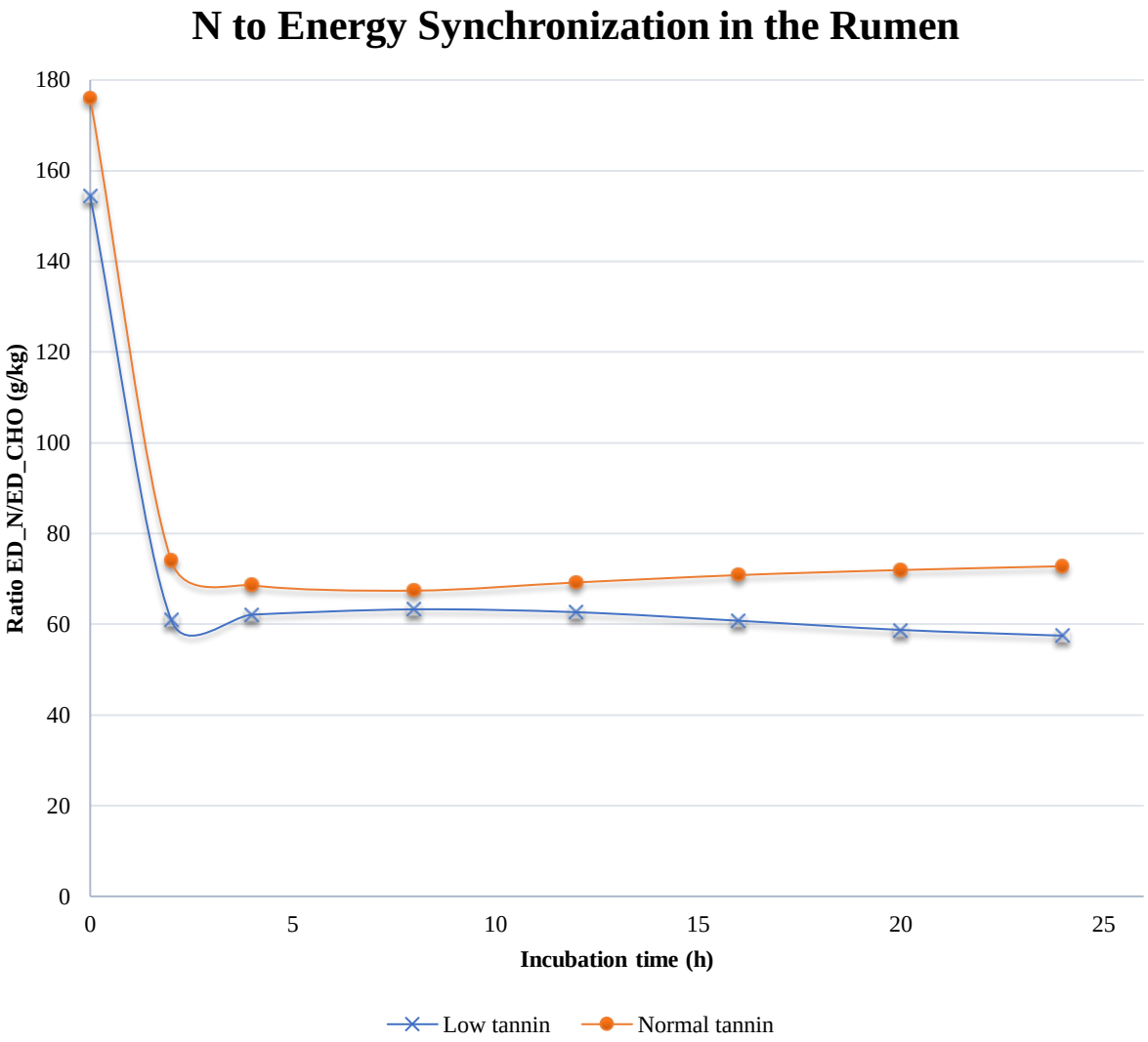


Figure 4.1. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of faba bean with low and normal tannin levels grown in western Canada.

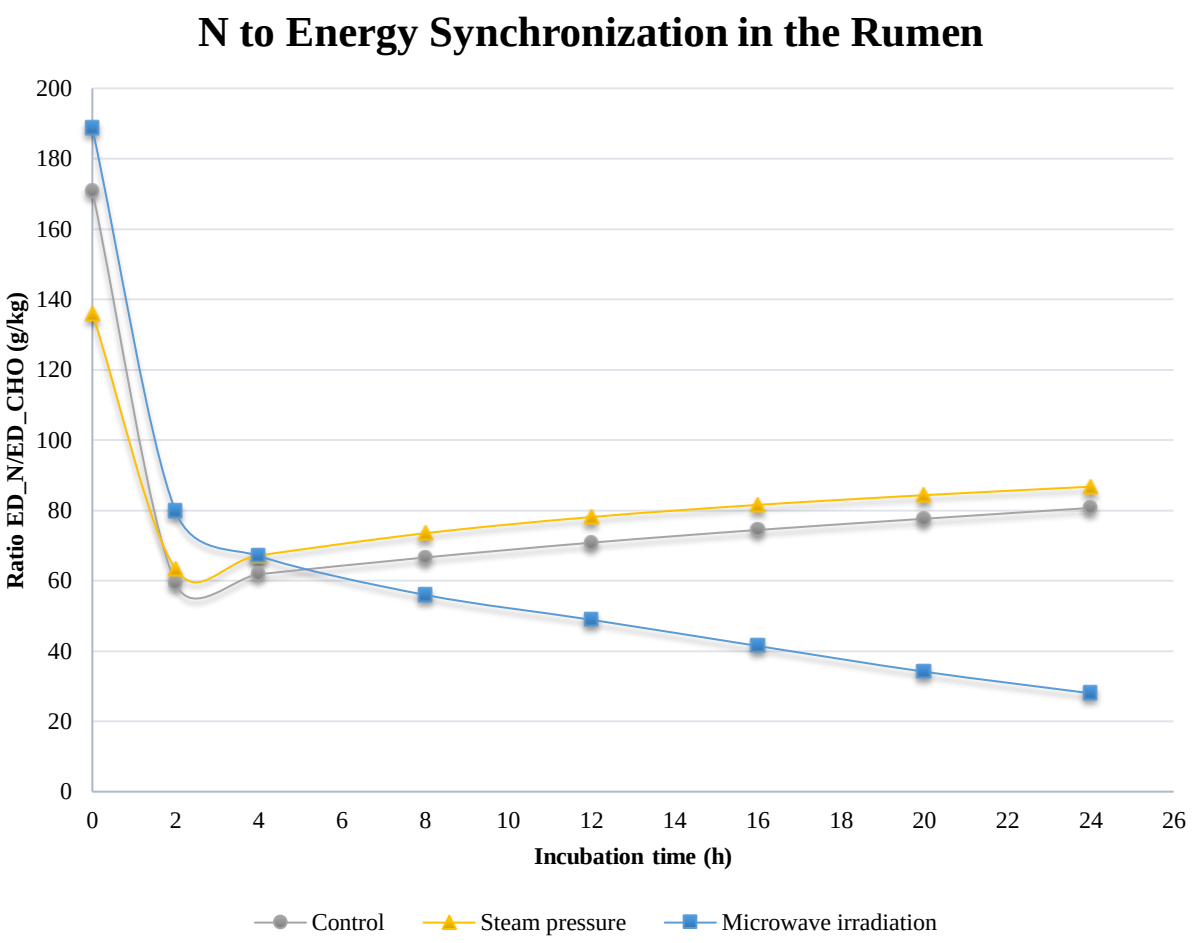


Figure 4.2. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

4.6.9. Effect of heat processing on prediction of truly digestible protein supply to the small intestine in dairy cattle with the DVE/OEB system of faba bean seeds with low and normal tannin level

Metabolic characteristics and true nutrient supply based on the DVE/OEB system are presented in Table 4.13. All metabolic characteristics showed significant differences ($P < 0.05$) due to treatment. The rumen undegraded crude protein (BCP) was higher in NT (+ 10 g/kg) than LT; SP increased BCP more (+ 87 g/kg) than MI and was also higher (+ 71 g/kg) than the Control. On the other hand, the effective degraded crude protein (EDCP), the microbial protein synthesized in the rumen based on available energy (MREE), the microbial protein synthesized in the rumen (MREN), and the rumen synthesized microbial protein truly absorbed in the small intestine (DVME) were reduced ($P < 0.05$) with SP (- 56, - 32, - 63, - 20 g/kg, respectively); MI did not change these values compared to the Control. In terms of truly absorbed bypass protein in the small intestine (DVBE), SP increased ($P < 0.05$) the amount (+ 70 g/kg) compared to the Control and + 84 g/kg compared to MI. The total true protein supply (DVE), degraded protein balance (OEB), and feed milk value were significant ($P < 0.05$) with both heat treatments. DVE and FMV was increased ($P < 0.05$) with SP (+ 50 g/kg, + 1 kg milk/kg feed, respectively) while MI reduced ($P < 0.05$) these values by 13 g/kg and 0.27 kg milk/kg feed respectively, compared to the Control.

Data from metabolic characteristics and true nutrient supply based on the NRC model are shown in Table 4.14. Significant differences ($P < 0.05$) with thermal treatments were revealed in all parameters. Rumen undegradable or bypass protein (RUP) and the truly absorbed microbial protein in the small intestine (AMCP) were higher ($P < 0.05$) in LT compared to NT. The seeds of faba bean showed double the amount of RUP and truly absorbed rumen undegradable protein in the small intestine (ARUP) with SP (126 and 112 g/kg, respectively) while MI reduced ($P < 0.05$) the

levels by 14 and 13 g/kg respectively, compared to the Control. However, the microbial protein synthesized in the rumen based on available TDN (MCP_{TDN}), the microbial protein synthesized in the rumen based on available protein (MCP_{RDP}), and the AMCP decreased with SP (- 3, - 48, - 2 g/kg, respectively) but remained the same with MI. The rumen endogenous protein (ECP) and the truly absorbed endogenous protein in the small intestine were greater ($P < 0.05$) than the Control with both heat treatments. The metabolizable protein (MP) and feed milk value (FMV) was increased with SP (+ 60 g/kg and + 1.23 kg milk/kg feed, respectively) while MI reduced their amount (- 14 g/kg and – 0.27 kg milk/kg feed, respectively). Similar outcomes were reported by Yu et al. (2004, 2005b) in which the DVE and MP values were higher due to a significant increase in the truly absorbed bypass protein which could compensate the lower microbial protein digested in the small intestine. Heat treatments in this study did not sufficiently reduce the OEB and DPB values in faba bean and levels are considered high enough to cause a potentially N loss in the rumen (Yu et al. 2004, 2005b).

Table 4.13. Metabolic characteristics and true nutrient supply (based on non-TDN system: DVE-OEB) of raw and heated faba bean seeds with low and high tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Truly digestible nutrient supply to dairy cows (g/kg DM)										
BCP	82[b]	92[a]	3.0	69[b]	140[a]	53[c]	3.7	0.04	<0.01	0.27
EDCP	202	201	3.3	218[a]	162[b]	225[a]	4.0	0.68	<0.01	0.24
MREE	112	109	1.2	121[a]	89[b]	122[a]	1.4	0.10	<0.01	0.28
MREN	194	191	3.5	211[a]	148[b]	220[a]	4.2	0.56	<0.01	0.22
DVME	71	70	0.7	77[a]	57[b]	78[a]	1.3	0.10	<0.01	0.28
DVBE	73	74	2.8	55[b]	125[a]	41[c]	3.4	0.67	<0.01	0.27
Degraded protein balance (OEB) and Total true protein supply (DVE) to dairy cows (g/kg DM)										
DVE	143	142	2.2	130[b]	180[a]	117[c]	2.8	0.92	<0.01	0.27
OEB	82	82	2.9	90[a]	59[b]	98[a]	3.5	0.99	<0.01	0.29
Feed Milk Value (kg milk/kg DM feed)										
FMV	2.90	2.89	0.046	2.65[b]	3.65[a]	2.38[c]	0.057	0.92	<0.01	0.29

Notes: [a-c] Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on available energy; EDCP: effective degradability of CP; MREN: microbial protein synthesized in the rumen; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

Table 4.14. Metabolic characteristics and true nutrient supply (based on TDN system: NRC dairy) of raw and heat treated faba bean seeds with low and high tannin levels grown in western Canada.

Item	Tannin level			Heat treatment				P value		
	Low (n= 9)	Normal (n= 9)	SEM	Control (n= 6)	Steam Pressure (n= 6)	Microwave Irradiation (n= 6)	SEM	Tannin level (T)	Heat treatment (Heat)	T × Heat
Truly digestible nutrient supply to dairy cows (g/kg DM)										
RUP	74^{b}	83^{a}	2.7	62^{b}	126^{a}	48^{c}	3.3	0.04	<0.01	0.27
MCP_{TDN}	100^{a}	99^{b}	0.4	101^{a}	98^{b}	100^{a}	0.5	0.03	<0.01	0.46
MCP_{RDP}	172	170	2.8	185^{a}	137^{b}	191^{a}	3.4	0.68	<0.01	0.24
AMCP	64^{a}	63^{b}	0.3	65^{a}	63^{b}	64^{a}	0.3	0.03	<0.01	0.46
ARUP	65	67	2.5	50^{b}	112^{a}	37^{c}	3.1	0.67	<0.01	0.27
ECP	11	11	0.0	11^{c}	11^{b}	12^{a}	0.0	0.17	<0.01	0.46
AECP	45	5	0.0	4^{c}	4^{b}	5^{a}	0.0	0.20	<0.01	0.48
Degraded protein balance (DPB) and Total metabolizable protein supply (MP) to dairy cows (g/kg DM)										
MP	134	135	2.7	119^{b}	179^{a}	105^{c}	3.3	0.85	<0.01	0.34
DPB	84	84	3.3	98^{a}	46^{b}	108^{a}	4.1	0.93	<0.01	0.30
Feed Milk Value (kg milk/kg DM feed)										
FMV	2.72	2.74	0.054	2.41^{b}	3.64^{a}	2.14^{c}	0.067	0.84	<0.01	0.34

Notes: $^{a\text{-}c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. RUP: rumen undegradable feed crude protein; MCP_{TDN}: rumen synthesized microbial protein base on available TDN; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; AMCP truly absorbed microbial protein in the small intestine; ARUP: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

4.7. Chapter summary and conclusions

Steam and microwave heat treatments affected the nutritional and metabolic characteristics of faba bean differently. Steam Pressure and Microwave Irradiation, increased the indigestible crude protein (PC) by 1.4 %, decreased the rapidly degradable CHO fraction (CA4), increased the intermediately degradable CHO fraction (CB1), and reduced the indigestible fiber fraction (CC). The intermediately degradable CHO fraction (CB2) was reduced only by Steam Pressure. In terms of rumen degradation of feed nutrients, the Kd (%/h) for DM was larger with Microwave Irradiation compared to the other treatments. Steam Pressure reduced the Kd (%/h) and soluble (S) fraction of DM, CP, and starch decreasing as a consequence the degradability of these nutrients (EDDM, EDCP and EDST). On the other hand, the degradable (D) fraction of CP and starch, additionally with the S fraction of NDF were increased with both thermal treatments. Steam Pressure however, produced a larger increase in RUP and BST while Microwave Irradiation reduced RUP and did not change BST. Goelema et al. (1998) state that the swelling capacity and solubility of starch is decreased with heat moisture treatments such as pressure toasting. The effects of MI on starch degradation parameters can be related to a lower moisture content during the process as Goelema et al. (1998) indicate that only treatments with steam at higher temperatures, or combined with mechanical process like flaking, improve the starch degradability. Both heat processing treatments decreased the content of $uNDF_{288h}$ in faba bean. Steam Pressure increased the intestinal digestibility of CP (dIDP), increased the intestinal digested crude protein (IADP), but also decreased the digestibility of bypass starch (dBST) and total digested starch (% TDST) compared to Microwave Irradiation and the Control. The gelatinized starch produce by heat after autoclaving can recrystallize during cooling periods producing an increase of resistant starch which is indigestible in the small intestine (Goelema et al. 1998). The metabolizable protein (MP),

the truly digested protein in the small intestine (DVE), and the feed milk value (FMV) were higher with the Steam Pressure treatment. On the other hand, Microwave Irradiation, had a larger effect on the fiber components and digestibility of rumen undegraded starch (dBST).

Low and normal tannin heat treated faba bean have different nutrient profiles, nutrient degradation and digestion characteristics, providing different nutrient supply to dairy cattle. As a consequence, thermal treatments change the metabolic characteristics of low and normal tannin faba bean. Based on this study, heat treatments shift the degradation parameters of main nutrients (DM, CP and starch). Steam Pressure is a suitable treatment to increase the rumen undegradable protein (RUP) and rumen undegradable starch (BST) while increasing the digestibility of protein in the small intestine. Furthermore, Steam Pressure could increase the true nutrient supply (MP) which improved the predicted production performance (FMV) of the different faba bean genotypes.

Heat processing had a significant impact on the nutrient supply to dairy cattle; however, different processing methods had different effects on faba bean. Steam Pressure was a better treatment for faba beans to improve the protein digestibility and increase the nutrient supply (MP, FMV) in dairy cattle in the present study.

5. MOLECULAR STRUCTURE SPECTRAL FEATURES AND HEAT PROCESSING INDUCED MOLECULAR STRUCTURE CHANGES IN DIFFERENT GENOTYPES OF FABA BEAN SEEDS WITH LOW AND NORMAL TANNIN LEVELS GROWN IN WESTERN CANADA, REVEALED USING VIBRATIONAL MOLECULAR SPECTROSCOPY.

5.1. Abstract

The aim of this study was to reveal the molecular structure spectral features of newly developed genotypes of faba bean seeds with low and normal tannin levels grown in western Canada and the molecular structure changes induced by heat processing methods using vibrational molecular spectroscopy. Additionally, the purpose of the analysis was to correlate the molecular structure parameters with the different nutrient and metabolic characteristics of the genotypes of faba bean. FTIR analyses were performed using a JASCO FT/IR–4200 spectroscope (JASCO Corp., Tokyo, Japan). Molecular structural features were analyzed in the mid-infrared region (ca. 4000–800 cm^{-1}) using the JASCO Spectra Manager II software (resolution of 4 cm^{-1}). Omnic 7.3 software (Spectra Tech, Madison, WI) was used to identify the different functional groups related to protein and carbohydrates molecular structure. Protein and carbohydrate structure spectral results were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, US). PROC CORR procedure was used for the correlation study and rank correlation was determined using the SPEARMAN test. PROC REG procedure for multi-regression analysis with a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05" was performed to FTIR data. Collinearity test was used with VIF option. Variables left in the regression model were significant at $\alpha = 0.05$ level.

Results showed a higher absorbance ($P < 0.05$) in normal tannin genotypes for amide I and amide II peak height and area, structural CHO (STCHO), total CHO (TCHO) area and peaks (H_1015, H_1076, H_1145) and cellulosic compounds (CEC) to total CHO (TCHO) ratio. Related to heat induced changes, Steam Pressure in low tannin genotypes increased the absorbance in amide I and amide II areas and α-helix peak height. Steam Pressure also increased the absorbance in amide I and amide II area, the absorbance in the structural CHO (STCHO) area, cellulosic compounds (CEC) area and peak height (H_1235). On the other hand, Microwave Irradiation increased the absorbance of all parameters related to total CHO spectral profiles, it reduced the absorbance in STCHO: TCHO ratio, and showed similar results to Control in the rest of parameters. Based on the previous data, significant correlations could be found between protein and CHO spectral profiles and chemical and metabolic characteristics of faba bean, which indicates that structural spectral features could be used to predict metabolic characteristics in animals. Additionally, FTIR analysis proved to be a useful tool to detect the responses of faba bean to heat processing methods by the different molecular structure changes induced by the treatments.

5.2. Introduction

Infrared spectroscopy (IR) is considered as a non-destructive and fast procedure for the analysis of chemical composition in feedstuffs. One great advantage of IR molecular spectroscopy is that practically any sample in any form can be analyzed. The speed and sensitivity of Fourier transform infrared spectroscopy (FTIR) makes it a good analysis technique in several science fields as relatively small amount of sample and little or no sample preparation are required. This method is cost-effective and reagent-free, avoiding the damage to feed components related to the use of several reagents in common chemical analysis.

The basis of this technique is related to the atom vibrations within a molecule. In IR spectroscopy, the identification of different chemical functional groups and spectra are obtained by passing infrared radiation through a sample to determine different molecule energy absorptions (Sun et al. 2018a; Stuart, 2004). Yang and Yu (2016), indicate that the infrared radiation absorption is proportional to the changes in energy due to different kinds of vibration in the molecules like bending or stretching. To detect inherent molecular structure of a specific sample, attenuated total reflectance Fourier transform infrared (ATR-FTIR) micro spectroscopy is usually used (Sun et al. 2018a). The IR region includes three main areas: near-IR (ca. 13,000 to 4,000 cm^{-1}), mid-IR (ca. 4,000 to 200 cm^{-1}), and far-IR (200 to 10 cm^{-1}); generally, the mid-IR region is the mostly used for analysing samples (Yang and Yu, 2016).

The main purpose of FTIR technology is to determine the particular molecular structure inherent to each feed ingredient which is highly associated with feed quality and its nutrient utilization by the animal. Information on chemical and structural characteristics of feedstuffs is valuable for breeding selection programs and prediction of quality and nutritive value of the feeds (Yu, 2005d).

5.3. Study objectives

1. To reveal molecular structure features of newly developed genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using molecular spectroscopic analysis.
2. To reveal heat processing induced molecular structure changes associated with nutrient utilization and availability in cattle using molecular spectroscopic analysis.
3. To determine the correlation between molecular structure features with nutrient profiles and physicochemical characteristics of faba bean grown in western Canada.

5.4. Study hypotheses

1. Molecular structure characteristics related to protein and carbohydrates spectral features could be significantly different between low and normal tannin genotypes.
2. Molecular structure features of protein and carbohydrates related spectra could be highly associated to nutrient profile, nutrient utilization and nutrient availability in dairy cattle.
3. Molecular changes of inherent structure induced by heat processing methods could be detected with Fourier transform infrared spectroscopy (FTIR).

5.5. Materials and Methods

5.5.1. Ingredients and sample preparation

All samples were finely ground through a 0.12 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) before performing the FTIR analysis.

5.5.2. Univariate molecular spectral analysis of protein and carbohydrate profiles

The FTIR analyses were performed at the College of Animal and Poultry Science in the University of Saskatchewan using a JASCO FT/IR–4200 spectroscope (JASCO Corp., Tokyo, Japan). The molecular structural features were analyzed in the mid-infrared region (ca. 4000–800 cm^{-1}). Five scans for each sample were collected using the JASCO Spectra Manager II software with a spectral resolution of 4 cm^{-1}. To identify the different functional groups related to protein and carbohydrates molecular structure, Omnic 7.3 software (Spectra Tech, Madison, WI) was used. Data from Huang et al. (2017) and Li et al. (2015) was used as the base to detect the several functional spectral bands of different faba bean used for this study. Spectral region for protein and polypeptides includes distinctive bands, amide I and amide II that result from the amide bonds that link amino acids (Figure 5.1.). Absorptions related with amide I leads to stretching vibrations of the amide C=O bond and absorptions associated with amide II leads to bending vibrations of the

N—H bond (Gallagher, 2009). Spectral profiles were analyzed in the following infrared regions: protein amide I and amide II (ca. 1718-1480 cm^{-1}), peak heights at ca. 1640 cm^{-1} and ca. 1435 cm^{-1}, respectively. Secondary protein structure α-helix and β-sheet were identified at peak heights ca. 1647 cm^{-1} and ca. 1627 cm^{-1}, respectively.

Carbohydrates related functional group region was observed at ca. 1481-942 cm^{-1}; spectral intensities of total carbohydrates (TCHO, ca. 1185 - 942 cm^{-1}), structural carbohydrates (STCHO, ca. 1481 - 1185 cm^{-1}), and cellulosic compounds (CEC, ca. 1288−1185 cm^{-1}) were determined. Absorption peak heights were found at ca. 1145 cm−1 (1st peak TCHO), ca. 1076 cm−1 (2nd peak TCHO), ca. 1015 cm−1 (3rd peak TCHO), ca. 1518 cm^{-1} (1^{st} peak STCHO), ca. 1445 cm^{-1} (2^{nd} peak STCHO) ca. 1390 cm^{-1} (3^{rd} peak STCHO), and ca. 1235 cm^{-1} (peak height CEC).

a) b)

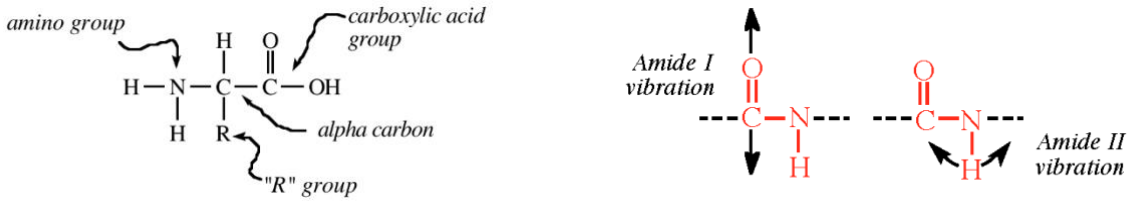


Figure 5.1. Peptide molecular structure and amide vibrations. a) amino acid structure. b) vibrations at amide I and amide II region. Source from Gallagher, 2009.

5.5.3. Correlation and multi regression analysis between molecular structure profiles and nutrient metabolic characteristics of protein and carbohydrates

Correlations between the molecular structure spectral data and chemical profiles, protein and carbohydrate fractions, energy values, *in situ* rumen degradation, intestinal digestion, and predicted protein supply were analyzed.

A multiple regression analysis was performed to select the best molecular spectral features that could explain the variation in chemical profiles, protein and carbohydrate fractions, energy values, *in situ* rumen degradation, intestinal digestion, and predicted protein supply.

5.5.4. Statistical analyses

Protein and carbohydrate structure spectral results were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, US). The model used was as follows:

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

where Y_{ij} was the observation of the dependent variable ij; μ was the population mean of the variable; T_i was the treatment effect (as a fixed effect); S was the subsample (5 scans per sample) which nested within each treatment; e_{ij} is the random error associated with the observation ij. Significance for the analyses were declared at $P < 0.05$ and trends at $P \leq 0.10$. Differences among the treatments were evaluated using the multiple treatment comparison Tukey method.

Associations between molecular structure spectral parameters and protein and carbohydrate related chemical composition, rumen degradation, intestinal digestion, and predicted protein supply were revealed by correlation and regression analyses in SAS 9.4. Normality of data was confirmed before performing further analysis. PROC CORR procedure was used for the correlation study and rank correlation was determined using the SPEARMAN test. In order to select relative variables of spectral parameters for predicting nutritional values, multiple regression study was conducted using PROC REG procedure with the following model: $Y = a + b_1 \times x_1 + b_2 \times x_2 + ... + b_n \times x_n$. The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". Collinearity test was performed using the VIF option to eliminate the influence of correlated dependent variables. All variables left in the final prediction model were significant

at α= 0.05 level. Residual analysis was performed using the Univariate procedure of SAS with Normal and Plot options.

5.5.5. Multivariate molecular spectral analysis of protein and carbohydrate profiles

Multivariate analysis implicates the analysis of many variables compared to a single one (univariate). PCA is an exploratory data analysis study and dimension-reduction tool that captures as closely as possible the variability of original variables by originating a data set with principal components (small number of independent linear combinations). On the other hand, CLA groups similar variables into a more representative group in which each element can represent an individual cluster, additionally, the cluster can be characterized by the most representative variable within each cluster (SAS institute, 2018). For the present study, multivariate analyses were determined using two statistic software including Statistica 8.0 Software (StatSoft Inc., Tulsa, OK, USA) and unscrambler analytics software 10.3 (CAMO Analytics Inc., Magnolia, TX, USA). CLA was analyzed using the Ward's method with squared Euclidean distance using Statistica 8.0.

5.6. Results and discussion

5.6.1. Fourier transform infrared spectroscopy (FTIR) analysis of protein, cellulosic compounds and carbohydrate profiles of newly developed genotypes of faba bean with low and normal tannin levels grown in western Canada.

5.6.1.1. Univariate analysis of protein related molecular structure spectral profiles

The protein structure is distinctive in its peptide bonds which are formed mainly by C=O, C–N and N–H. Two main amide components related to the protein region are usually studied in nutrient feed analysis. Protein identification is mainly related to the amide I as amide II is usually overlapped with other structural bands (Mostafizar, 2017). Representation of a typical FTIR spectra analysis is presented in Figure 5.1. In this study, significant differences ($P < 0.05$) were

observed in the protein molecular structure spectral profiles of different genotypes of faba bean (Table 5.2.). NT showed higher ($P < 0.05$) absorbance units (AU) in amide I and amide II peak area and height, and in β-sheet peak height while α-helix was not significant ($P > 0.10$) between tannin levels. Total amide area was higher ($P < 0.05$) in 346_10 compared to Vertigo and FB9_4 which were not different from other genotypes. 346_10 presented a higher ($P < 0.05$) absorbance in amide I, amide II areas (26.87, 12.33 AU, respectively) and heights (0.416, 0.239 AU, respectively) and α-helix and β-sheet peak heights (0.500 and 0.456 AU, respectively). However, 346_10 was not different from Snowdrop, 219_16, FB9_4, and SSNS_1 in terms of amide II peak height and β-sheet peak height. Vertigo showed the lower absorbance in total amide area (54.52 AU) but was not different from Snowbird, 219_16, Fatima and SSNS_1. The area ratio of amide I to amide II and the height ratio of α-helix to β-sheet was higher ($P < 0.05$) in LT compared to NT while the height ratio of amide I to amide II was not significantly different ($P > 0.10$) among genotypes and between tannin levels. Amide I: amide II area was higher in Snowbird (2.326 AU) and Vertigo (2.314 AU) while the remained genotypes were similar to each other. In terms of α-helix: β-sheet peak height, the greater ($P < 0.05$) values were shown in Snowbird and 219_16 (1.111 and 1.110 AU, respectively) but were not different from other genotypes except for FB9_4. Carbonaro et al. (2014) stated that a large β-sheet and low α-helix component characterize the protein structure of legume seeds and other plant proteins such as cereal grains. Similar results were found in Wang et al. (2013) in which amide I was around 1654 cm^{-1} (mainly related to C=O stretching vibration) and amide II near 1538 cm^{-1} (linked to N-H bending vibrations and C-N stretching vibrations). Wang et al. (2013) stated that the nutrient difference between white and green broad beans is possibly caused by the difference on their secondary protein structure.

Table 5.1. Protein molecular structure spectral profiles of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using Fourier transform infrared attenuated total reflectance molecular spectroscopy (FTIR).

		Low tannin genotypes (LT)			Normal tannin genotypes (NT)							Contrast P value
Item	peak region and center (cm^{-1})	Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1	SEM	P value	LT vs. NT
Protein related primary structure												
total amide	ca.1480-1718	56.10^{bc}	56.97^{b}	55.50^{bc}	56.46^{bc}	54.52^{c}	57.16^{b}	59.40^{a}	55.77^{bc}	1.096	<0.01	0.21
amide I area		25.15^{b}	25.58^{b}	25.07^{b}	25.65^{b}	24.74^{b}	25.41^{b}	26.87^{a}	25.56^{b}	0.628	<0.01	0.02
amide II area		10.83^{cd}	11.41^{bc}	11.42^{bc}	11.69^{b}	10.69^{d}	11.6^{b}	12.33^{a}	11.52^{b}	0.214	<0.01	<0.01
amide I peak height	ca.1640	0.391^{b}	0.397^{b}	0.390^{b}	0.398^{b}	0.386^{b}	0.395^{b}	0.416^{a}	0.396^{b}	0.0083	<0.01	0.03
amide II peak height	ca.1534	0.208^{c}	0.221^{abc}	0.222^{abc}	0.219^{bc}	0.214^{bc}	0.228^{ab}	0.239^{a}	0.224^{abc}	0.0054	<0.01	0.02
Protein related secondary structure												
α-helix peak height	ca.1647	0.477^{b}	0.481^{b}	0.470^{b}	0.478^{b}	0.466^{b}	0.478^{b}	0.500^{a}	0.472^{b}	0.0100	<0.01	0.28
β-sheet peak height	ca.1627	0.429^{b}	0.438^{ab}	0.423^{b}	0.434^{b}	0.424^{b}	0.438^{ab}	0.456^{a}	0.430^{b}	0.0089	<0.01	0.04
Ratios												
amide I:II area		2.326^{a}	2.245^{b}	2.197^{b}	2.195^{b}	2.314^{a}	2.191^{b}	2.180^{b}	2.222^{b}	0.0263	<0.01	<0.01
amide I:II height		1.883	1.795	1.756	1.948	1.809	1.733	1.741	1.768	0.0773	0.47	0.84
α-helix: β-sheet height		1.111^{a}	1.098^{ab}	1.110^{a}	1.103^{ab}	1.100^{ab}	1.092^{b}	1.096^{ab}	1.098^{ab}	0.0047	0.01	<0.01

Notes: $^{a-d}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. The peak area and the peak height presented in each functional group measurements were expressed in IR absorbance units.

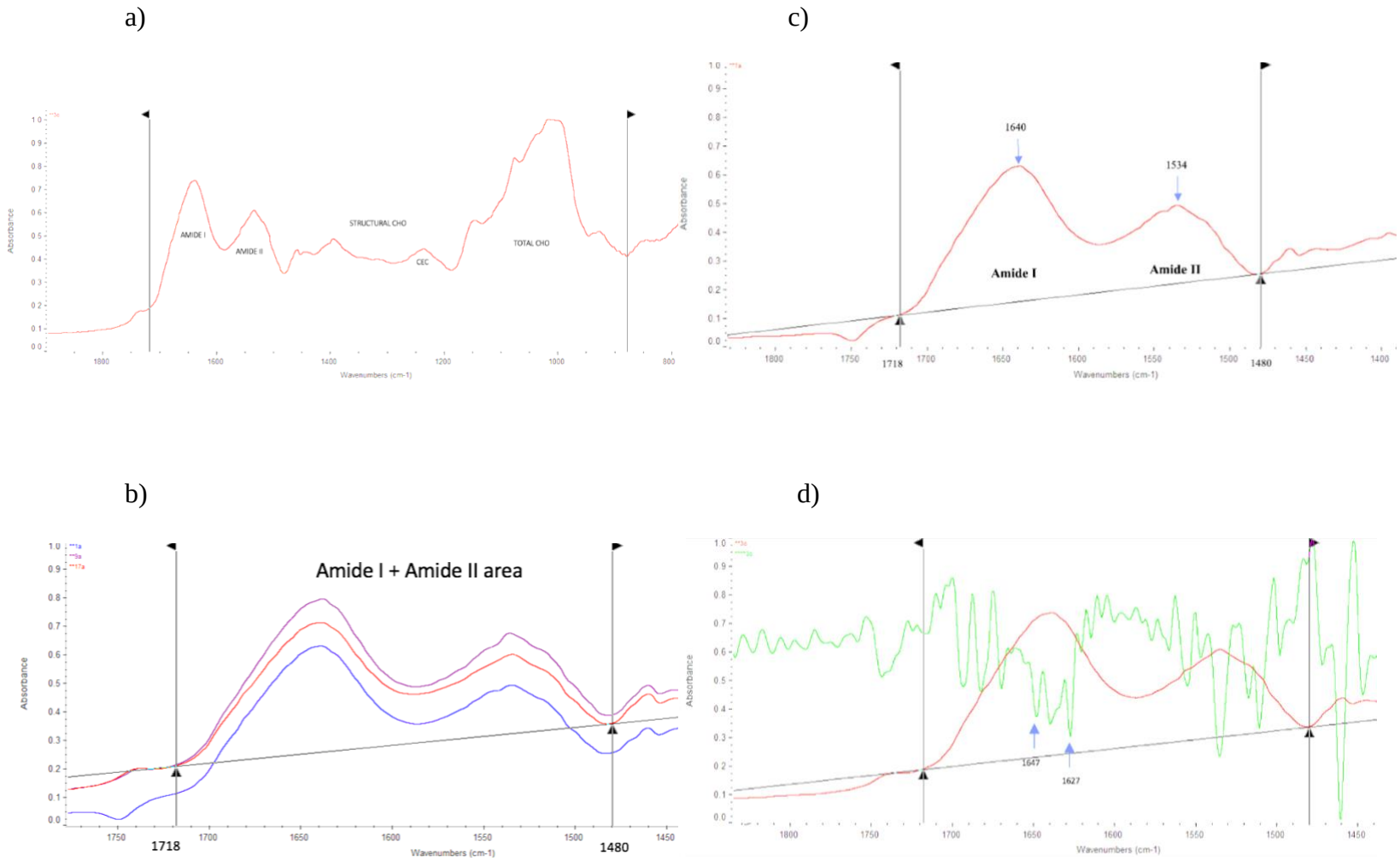


Figure 5.2. Typical FTIR spectrum from different genotypes of faba bean seeds grown in western Canada at protein amide I and amide II related functional group region (ca. 1718-1480 cm^{-1}). a) protein and CHO related functional group area; b) amide related functional group area from low tannin faba bean (Snowbird) from three different locations; c) protein primary structure: amide I peak height and amide; d) protein secondary structure α-helix and β-sheet peak height.

5.6.1.2. Univariate analysis of carbohydrates and cellulosic compounds related molecular structure spectral profiles

Carbohydrates are characterized by the existence of CO and OH bonds. Absorptions could be found around 3000 to 2800 cm^{-1} (mainly C–H stretching), and around 1200 to 800 cm^{-1} (related to C=O and C–C stretching vibrations and C–O–H deformation) (Mostafizar, 2107). Spectra data related to CHO molecular structure is shown in Figure 5.2.

The molecular structure spectral characteristics of carbohydrates of different genotypes of faba bean are presented in Table 5.3. Parameters related to structural carbohydrates (STCHO), cellulosic compounds (CEC), and total carbohydrates (CHO) were analyzed. The area of STCHO and CHO (20.52 and 68.63 AU, respectively) and the height of CHO peak 1, peak 2, and peak 3 (0.187, 0.408 and 0.532 AU, respectively) were higher ($P < 0.05$) in NT compared to LT. The higher values for STCHO area were observed in FB9_4 (20.97 AU) and SSNS_1 (20.99 AU) but these genotypes were different from 346_10 (19.81 AU) only. The height of the 1st STCHO peak related to lignin molecular structure (ca. 1518 cm^{-1}) showed less absorbance in Fatima (0.011 AU) which was similar to the rest of samples except for FB9_4 (0.017 AU). The 2nd STCHO peak (1445 cm^{-1}) presented a higher absorbance in 219_16, Fatima, Vertigo, 346_10, and SSNS_1, but showed similar results to Snowbird and Snowdrop. Finally, the 3rd STCHO peak (1390 cm^{-1}) was greater ($P < 0.05$) in SSNS_1 (0.136 AU), but showed no difference with Snowbird, Snowdrop, Fatima, Vertigo, and FB9_4. Larger values related to CEC area were observed in Snowbird and SSNS_1 (3.64 and 3.63 AU, respectively), lower absorbance in Vertigo (3.39 AU); however, Snowbird and SSNS_1 were not different from the other genotypes. Peak height of CEC was higher in Snowdrop and 346_10 (0.076 AU for both) and they were not different from the other genotypes, excluding 219_16 (0.070 AU). The total CHO related molecular structure profiles showed a greater CHO

area in the variety FB9_4 (70.08 AU) but it was not different from Snowdrop, Fatima, Vertigo, and SSNS_1. FB4_9 showed higher 1st and 3rd CHO peak heights (0.191 and 0.551 AU) compared to other genotypes; however, the height of CHO peak 1 was similar between FB9_4, Snowdrop, Fatima, Vertigo, and SSNS_1. Total CHO peak 2 height was higher in Vertigo (0.415 AU) and this was not different from all the other genotypes except for Fatima (0.400 AU). The area ratio of CEC to CHO presented a lower value in Vertigo (0.049 AU) and was similar Snowdrop, 219_16, and FB9_4. On the other hand, the higher result related to the area ratio of CEC to STCHO was observed in 346_10 which was not different from Snowbird, Snowdrop, Fatima, and SSNS_1.

a)

b)

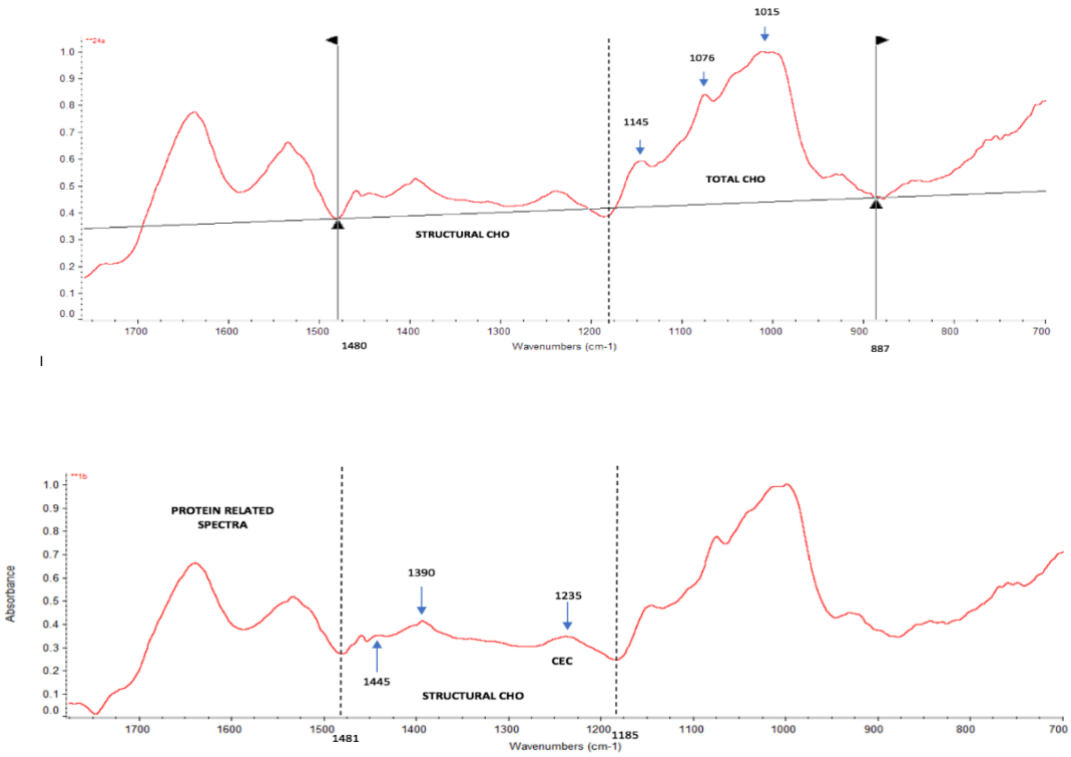


Figure 5.3. Typical FTIR spectrum from different genotypes of faba bean seeds grown in western Canada at carbohydrate related functional group region (ca. 1481-887 cm^{-1}). a) total CHO area and related peaks (peak area region and baseline, ca. 1185 - 942 cm^{-1}) of normal tannin faba bean SSNS_1; b) structural CHO and related peaks (peak area region and baseline, ca. 1481 - 1185 cm^{-1}) from low tannin faba bean (Snowbird).

Table 5.2. Carbohydrate molecular structure spectral profiles of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using Fourier transform infrared attenuated total reflectance molecular spectroscopy (FTIR).

Item	peak region and center (cm^{-1})	Low tannin genotypes (LT)			Normal tannin genotypes (NT)					SEM	Contrast P value	
		Snowbird	Snowdrop	219_16	Fatima	Vertigo	FB9_4	346_10	SSNS_1		P value	LT vs. NT
Structural carbohydrates (STCHO) related to spectral profile												
1st peak height	ca. 1518	0.014^{ab}	0.014^{ab}	0.013^{ab}	0.011^{b}	0.013^{ab}	0.017^{a}	0.013^{ab}	0.015^{ab}	0.0012	0.02	0.80
2nd peak height	ca.1445	0.091^{ab}	0.091^{ab}	0.091^{a}	0.091^{a}	0.092^{a}	0.088^{b}	0.092^{a}	0.092^{a}	0.0008	<0.01	1.00
3rd peak height	ca.1390	0.132^{abc}	0.132^{abc}	0.126^{c}	0.131^{abc}	0.131^{abc}	0.133^{ab}	0.128^{bc}	0.136^{a}	0.0016	<0.01	0.09
STCHO area	ca. 1481 - 1185	20.08^{ab}	20.22^{ab}	19.97^{ab}	20.57^{ab}	20.28^{ab}	20.97^{a}	19.81^{b}	20.99^{a}	0.327	0.01	0.02
Cellulosic compounds (CEC) related to spectral profile												
CEC peak height	ca.1235	0.076^{a}	0.074^{ab}	0.070^{b}	0.073^{ab}	0.072^{ab}	0.074^{ab}	0.076^{a}	0.073^{ab}	0.0011	<0.01	0.85
CEC area	ca. 1288−1185	3.64^{a}	3.52^{ab}	3.40^{ab}	3.59^{ab}	3.39^{b}	3.60^{ab}	3.61^{ab}	3.63^{a}	0.064	<0.01	0.24
Total carbohydrates (TCHO) related to spectral profile												
1st peak height	ca.1145	0.184^{b}	0.187^{ab}	0.185^{b}	0.187^{ab}	0.187^{ab}	0.191^{a}	0.184^{b}	0.187^{ab}	0.0012	<0.01	0.03
2nd peak height	ca.1076	0.403^{ab}	0.403^{ab}	0.401^{ab}	0.400^{b}	0.415^{a}	0.413^{ab}	0.403^{ab}	0.408^{ab}	0.0036	0.01	0.04
3rd peak height	ca.1015	0.522^{bc}	0.527^{bc}	0.524^{bc}	0.530^{bc}	0.541^{ab}	0.551^{a}	0.513^{c}	0.527^{bc}	0.0054	<0.01	0.02
TCHO area	ca. 1185 - 942	67.77^{bc}	68.07^{abc}	67.58^{c}	67.94^{abc}	69.85^{ab}	70.08^{a}	67.12^{c}	68.18^{abc}	0.615	<0.01	0.03
Area ratios related to spectral profile												
STCHO: TC		0.296	0.298	0.296	0.303	0.290	0.300	0.295	0.308	0.0047	0.11	0.37
CEC: TCHO		0.054^{a}	0.052^{ab}	0.050^{ab}	0.053^{a}	0.049^{b}	0.051^{ab}	0.054^{a}	0.053^{a}	0.0008	<0.01	0.86
CEC: STCHO		0.181^{ab}	0.174^{abc}	0.170^{c}	0.175^{abc}	0.167^{c}	0.172^{bc}	0.182^{a}	0.173^{abc}	0.0024	<0.01	0.43

Notes: a-c Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. STCHO (peak area region and baseline, ca. 1481 - 1185 cm^{-1}); TCHO (peak area region and baseline, ca. 1185 - 942 cm^{-1}); CEC (peak area region and baseline, ca. 1288−1185 cm^{-1}). The peak area and the peak height presented in each functional group measurements were expressed in IR absorbance units.

5.6.1.3. Correlation analysis between protein related molecular structure features with protein profiles and metabolic characteristics.

A correlation analysis was performed to determine the relationship between protein structural characteristics and protein bioavailability of different genotypes of faba bean grown in western Canada. Detailed data from the study is presented in Table 5.3. Crude protein (CP) was positively correlated ($P < 0.05$) with total amide ($r = 0.54$), amide I ($r = 0.56$), amide II ($r = 0.66$) areas, and also with peak heights of amide I ($r = 0.49$), amide II ($r = 0.66$), α-helix ($r = 0.52$), and β-sheet ($r = 0.54$), so when these molecular structures increased in faba bean the protein content would proportionally increase as well. On the other hand, negative relationships were also observed between the variables, meaning that when a higher content of a first variable is presented a lower level in the second variable would be observed, and vice versa. CP was negatively correlated ($P < 0.05$) with the ratio of amide I to amide II peak height ($r = - 0.42$). NDICP was negatively correlated to amide I to amide II area ratio and height ($r = - 0.57$ and $r = - 0.56$, respectively). Non-protein nitrogen (NPN) was positively correlated ($P < 0.05$) to amide I area ($r = 0.46$), amide I peak height ($r = 0.44$), and α-helix peak height ($r = 0.48$). These results agreed with published data from Xin et al. (2016) in which authors observed a relationship between protein spectral features and nutrient profiles in different feed by-products. In regard to the slowly degradable protein fraction (PB2), a negative correlation with the area and height ratios of amide I to amide II ($r = - 50$ and $r = - 49$, respectively) was observed. The rapidly degradable protein fraction (PA2) tended to a positive correlation ($P < 0.10$) with amide I to amide II area ratio but did not show any association with the other molecular features. Soluble crude protein, moderately degradable protein fraction (PB1), undegradable protein fraction (PC), and true protein were not significantly correlated with the spectral parameters.

Association between protein related molecular structure and different energy characteristics was observed and it is represented in Table 5.4. The truly digestible crude protein (tdCP) was positively correlated ($P < 0.05$) with most of the molecular features: total amide ($r = 0.54$), amide I ($r = 0.56$), amide II ($r = 0.66$) areas, and also with peak heights of amide I ($r = 0.49$), amide II ($r = 0.66$), α-helix ($r = 0.52$), and β-sheet ($r = 0.54$) and was negatively correlated with amide I to amide II peak height ratio ($r = - 0.42$). Digestible energy at 1-time maintenance (DE_{1x}) and digestible energy for production at 3 times maintenance (DEp_{3x}) had a positive correlation ($P <0.05$) with amide II ($r = 0.42$ and $r = 0.45$, respectively) area and height ($r = 0.44$ and $r = 0.46$, respectively). Digestible energy (DEp_{3x}) showed a negative correlation ($P < 0.05$) with α-helix to β-sheet peak height ratio ($r = - 0.42$). Metabolizable energy tended to be correlated ($P < 0.10$) with amide II area and height, as well as α-helix to β-sheet peak height ratio. Net energy of lactation (NE_L) was positively correlated with amide II height ($r = 0.41$) and tended ($P < 0.10$) to present a positive correlation with amide II area and a negative correlation with the height ratio of α-helix to β-sheet. ME based on NRC beef and net energy of maintenance (NE_m) were both positively correlated with amide II area and amide II height, and negatively correlated with α-helix to β-sheet peak ratio. A tendency ($P < 0.10$) was observed in amide I to amide II area ratio and β-sheet peak height in both ME-beef and NE_m. Net energy of gain (NE_g) showed a negative correlation as well with α-helix to β-sheet peak ratio ($r = - 0.44$); this parameter tended to have a negative and positive relationship with the ratio of amide I to amide II area and amide II peak height, respectively ($P < 0.10$). Rumen protein degradation parameters such as rumen undegradable crude protein (BCP and RUP) were negatively correlated ($P < 0.05$) with amide I to amide II area ratio ($r = -0.43$), amide I to amide II peak height ($r = -0.44$), and α-helix to β-sheet peak ratio ($r = -0.43$). The effectively degraded crude protein (EDCP) correlated positively with most of the molecular structure features: total amide

area (r = 0.49), amide I area (r = 0.44), amide II area (r = 0.54), amide I peak height (r = 0.45), amide II peak height (r = 0.54), α-helix (r = 0.49), and β-sheet (r = 0.43).

Table 5.5. shows the association between protein structural characteristics and intestinal protein digestion of different genotypes of faba bean grown in western Canada. The intestinal digestibility of protein (dIDP) showed a positive correlation with the ratios of amide I to amide II area (r = 0.45) and α-helix to β-sheet peak height (r = 0.46). The total digested protein (TDP) was positively correlated with total amide area (r = 0.60), amide I area (r = 0.58), amide II area (r = 0.64), amide I peak height (r = 0.53), amide II peak height (r = 0.65), α-helix (r = 0.58), and β-sheet (r = 0.56). The truly digested protein in the small intestine (DVE), metabolizable protein (MP), and feed milk value (FMV) showed no correlation among parameters; however, the degraded protein balance (OEB and DPB) had a positive correlation with the total amide, amide I, and amide II areas, as well as the amide I, amide II, α-helix, and β-sheet peak heights. β-sheet structure has been widely related with a lower digestibility of protein; previous studies have shown a negative correlation between the content of β-sheet and protein digestibility (Yu, 2005c; Refat, 2018; Carbonaro, 2012). Little information is available on correlation data between molecular spectral features and metabolic characteristics of faba bean; therefore, data related to other feeds including cereals and other legumes were used for matter of comparison in this research.

Correlation results in the present study proved to be useful to estimate the metabolic characteristics of faba bean grown in western Canada. The high solubility and degradability of the seeds could be possibly related to the positive correlation between β-sheet related structures and protein features which could also be the cause for the significant relationship found between protein related molecular structures and the degraded protein balance (OEB).

Table 5.3. Correlation analyses between protein structure spectral characteristics and protein profiles, protein sub-fractions of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Basic protein profiles										
CP (%DM)	0.56**	0.66**	-0.28	0.54*	0.49*	0.66**	-0.42*	0.52*	0.54*	-0.26
NDICP (%CP)	0.04	0.22	-0.57**	-0.03	0.02	0.22	-0.56**	-0.06	0.05	-0.39+
ADICP (%CP)	0.17	0.05	-0.01	0.12	0.23	-0.05	0.10	0.17	0.17	-0.06
SCP (%CP)	0.18	0.15	0.21	0.25	0.16	0.22	0.07	0.25	0.15	0.17
NPN (%CP)	0.46*	0.30	0.27	0.39+	0.44*	0.31	0.24	0.48*	0.32	0.19
Protein sub-fractions										
PA2 (%CP)	0.02	-0.08	0.36+	0.10	0.02	0.00	0.26	0.10	-0.03	0.30
PB1 (%CP)	0.02	0.08	-0.29	-0.05	0.04	-0.04	-0.15	-0.04	0.09	-0.29
PB2 (%CP)	-0.01	0.14	-0.50*	-0.08	0.00	0.15	-0.49*	-0.11	0.00	-0.33
PC (%CP)	0.15	0.03	-0.04	0.10	0.20	-0.06	0.08	0.13	0.13	-0.05
TP (%CP)	-0.15	-0.03	0.04	-0.10	-0.20	0.06	-0.08	-0.13	-0.13	0.05
PA2tp (%CP)	0.02	-0.08	0.36+	0.10	0.02	0.00	0.26	0.10	-0.03	0.30
PB1tp (%CP)	0.01	0.07	-0.28	-0.06	0.03	-0.05	-0.14	-0.05	0.07	-0.27
PB2tp (%CP)	-0.02	0.14	-0.51*	-0.09	-0.01	0.14	-0.48*	-0.12	-0.01	-0.33

Notes: DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; PA2: rapidly degradable protein fraction; PB1: moderately degradable protein fraction; PB2: slowly degradable protein fraction; PC: unavailable protein; tp = true protein; "+": P<0.10, not significant with tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

Table 5.4. Correlation analyses between protein structure spectral characteristics and estimated energy profiles, rumen protein degradation of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Estimated energy profiles										
tdCP (%DM)	0.56**	0.66**	-0.28	0.54*	0.49*	0.66**	-0.42*	0.52*	0.54*	-0.26
TDN1x (%DM)	-0.01	0.11	-0.28	0.07	0.01	0.15	-0.12	0.05	0.09	-0.34+
DE_{1x} (Mcal/kg)	0.28	0.42*	-0.31	0.32	0.25	0.44*	-0.26	0.30	0.32	-0.32
DEp_{3x} (Mcal/kg)	0.28	0.45*	-0.40+	0.32	0.24	0.46*	-0.33	0.28	0.33	-0.42*
ME_dairy (Mcal/kg)	0.24	0.38+	-0.30	0.28	0.22	0.41+	-0.25	0.26	0.30	-0.35+
NE_L (Mcal/kg)	0.27	0.41+	-0.31	0.30	0.23	0.41*	-0.27	0.27	0.33	-0.37+
ME_beef (Mcal/kg)	0.33	0.47*	-0.38+	0.37+	0.28	0.49*	-0.30	0.34	0.37+	-0.43*
NE_m (Mcal/kg)	0.28	0.43*	-0.38+	0.34	0.25	0.44*	-0.31	0.31	0.35+	-0.42*
NE_g (Mcal/kg)	0.21	0.39	-0.39+	0.26	0.19	0.41+	-0.30	0.23	0.29	-0.44*
Rumen protein degradation										
BCP^{DVE} (g/kg DM)	0.13	0.28	-0.43*	0.08	0.09	0.26	-0.44*	0.04	0.23	-0.43*
RUP^{NRC} (g/kg DM)	0.13	0.28	-0.43*	0.08	0.09	0.26	-0.44*	0.04	0.23	-0.43*
EDCP (g/kg DM)	0.44*	0.54*	-0.11	0.49*	0.45*	0.54*	-0.15	0.49*	0.43*	-0.15

Notes: tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one time maintenance. $DE_{1\times}$: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for growth; BCP^{DVE}: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; "+": P<0.10, not significant with tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

Table 5.5. Correlation analyses between protein structure spectral characteristics and intestinal protein digestion, predicted truly absorbed protein supply of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Intestinal protein digestion										
dIDP (%)	0.14	0.00	0.38^{+}	0.16	0.12	-0.08	0.45*	0.22	0.04	0.46*
IDP (g/kg DM)	0.24	0.25	-0.10	0.21	0.19	0.20	-0.14	0.20	0.28	-0.10
TDP (g/kg DM)	0.58**	0.64**	-0.18	0.60**	0.53*	0.65**	-0.28	0.58**	0.56**	-0.16
DVE/OEB model										
DVE (g/kg DM)	0.31	0.31	-0.12	0.28	0.25	0.21	-0.09	0.28	0.33	-0.09
OEB (g/kg DM)	0.45*	0.56**	-0.09	0.49*	0.45*	0.55*	-0.16	0.48*	0.42*	-0.09
FMV (kg milk/kg	0.30	0.31	-0.13	0.27	0.25	0.22	-0.11	0.27	0.32	-0.09
NRC values										
MP (g/kg DM)	0.21	0.22	-0.12	0.19	0.16	0.20	-0.15	0.17	0.26	-0.15
DPB (g/kg DM)	0.47*	0.54*	-0.07	0.51*	0.47*	0.54*	-0.13	0.52*	0.45*	-0.11
FMV (kg milk/kg	0.23	0.26	-0.14	0.21	0.18	0.23	-0.15	0.19	0.28	-0.16

Notes: dIDP: intestinal digestibility of crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value in DVE/OEB system; MP: metabolizable protein; DPB: rumen degraded protein balance; "$^{+}$": P<0.10, not significant with tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

5.6.1.4. Correlation analysis between carbohydrates (CHO) related molecular structure features with CHO profiles and metabolic characteristics

In Table 5.6. results for correlation analyses from CHO molecular structure characteristics, CHO profiles, and CHO fractions are presented. CHO nutrient profile was positively correlated with the total CHO area (TCHO) (r = 0.44) and 2nd CHO peak (r = 0.43) in the molecular spectra, which implies that when higher intensities in TCHO greater content of total CHO could be probably found in faba bean seeds. Sugar was negatively correlated with CHO area (r = - 0.67), CHO peak 2 (r = - 0.47) and CHO peak 3 (r = - 0.69) and tended to have a negative correlation with CHO peak 1. Additionally, a positive association was found between sugar and CEC to CHO ratio (r = 0.46). Neutral detergent fiber (NDF) was not significantly correlated with any of the parameters; however, tended to be significantly (P < 0.10) related to the structural carbohydrates (STCHO) and STCHO peak 3. The acid detergent fiber (ADF) had a positive correlation with STCHO and STCHO peak 3; also, a tendency was observed STCHO peak 2, area related to cellulosic compounds (CEC), and 1st peak of CHO (P < 0.10). Cellulose showed a positive connection with the 3rd STCHO peak (r = 0.44) and tendency (P < 0.10) with the area of CEC. No correlation was observed between non-structural CHO (NSC), acid detergent lignin (ADL), hemicellulose, and uNDF. Data related to CHO sub-fractions showed a negative correlation between the slowly degradable carbohydrate fraction (CB3) and the ratio of CEC to STCHO (r = - 0.46).

Results from the correlation analysis from the molecular structure of CHO with energy values and rumen degradation characteristics are presented in Table 5.7. A significant correlation (P < 0.05) was observed between metabolizable energy (ME) based on NRC dairy and CHO peak 2 (r = - 0.41); rumen undegradable NDF showed association with the 3rd STCHO peak (r = 0.44). The effective degraded crude protein (EDCP) showed a negative correlation with CHO area and CHO

2nd peak; however, a tendency was observed in the 3rd CHO peak ($P < 0.10$) as well. Tendencies ($P < 0.10$) were found between the following parameters: truly digestible non-fiber carbohydrates (tdNFC) and CHO area, DE_{1x} with CHO area and CHO 2nd peak, $DEp_{3x,}$ NE_m, and NE_g with CHO 2nd peak, effective degraded neutral detergent fiber (EDNDF) and CEC to STCHO ratio ($r = 0.36$) and finally rumen undegraded or bypass starch (BST) with STCHO 2nd peak ($r = 0.40$).

Data from the association between carbohydrates (CHO) related molecular structure profiles and intestinal digestion parameters are shown in Table 5.8. Total digested neutral detergent fiber (TDNDF) had a tendency ($P < 0.10$) to be correlated to only one of the parameters related to molecular structure features (3rd CHO peak); total digested starch did not show any associations. The intestinal digestibility of protein (dIDP) tended to have a negative correlation ($P < 0.10$) with STCHO area and 3rd STCHO peak height. The intestinal digestible protein (IADP) was negatively correlated with the cellulosic compounds (CEC) area ($r = - 0.40$). Other negative correlations were found between: total digested protein (TDP) in the small intestine and the CHO area ($r = - 0.47$) and 2nd CHO peak height ($r = - 0.42$) and degraded protein balance (OEB and DPB) with CHO area and 2nd CHO peak height. The truly digested protein in the small intestine (DVE), feed milk value based on DVE/OEB system (FMV_{DVE}), and rumen undegraded or bypass protein based on the NRC model (RUP) did not show any correlations with the CHO molecular structure parameters. These results agreed with Xin et al. (2013) who found no significant correlations between the molecular spectral intensities of STCHO peak area and all the nutritive features in *Brassica carinata* and canola seeds. However, data in this study are not fully in agreement with published data performed in agricultural by-products (Xin et al. 2016).

Table 5.6. Correlation analyses between carbohydrate structure spectral characteristics and carbohydrate profiles, carbohydrate sub-fractions of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCH: TCHO	CEC: TCHO	CEC: STCHO
Basic nutrient profiles (%DM)													
CHO	0.27	0.01	0.18	0.24	0.26	0.19	0.44*	0.20	0.43*	0.31	0.08	0.08	-0.01
Starch	-0.11	-0.08	0.02	-0.18	-0.14	-0.07	-0.02	0.02	-0.04	-0.02	-0.10	0.03	0.06
NFC	0.05	-0.20	-0.12	0.04	0.19	0.14	0.34	0.08	0.17	0.26	-0.04	0.07	0.17
Sugar	0.07	0.02	0.21	0.17	0.17	0.10	-0.67**	-0.41+	-0.47*	-0.69**	0.24	0.46*	0.19
NSC	-0.07	-0.04	0.07	-0.15	-0.10	-0.02	-0.11	-0.06	-0.07	-0.14	-0.06	0.10	0.07
NDF	0.36+	0.26	0.14	0.36+	0.14	0.10	0.13	0.24	0.18	0.18	0.21	0.04	-0.24
ADF	0.41*	0.20	0.36+	0.53*	0.40+	0.33	0.17	0.41+	0.23	0.17	0.32	0.28	-0.07
ADL	0.16	-0.01	0.21	0.33	0.24	0.17	0.09	0.23	0.10	0.07	0.21	0.17	0.06
Hemicellulose	0.18	0.12	0.10	0.10	-0.05	-0.07	0.16	0.10	0.17	0.17	0.05	-0.13	-0.24
Cellulose	0.34	0.27	0.27	0.44*	0.37+	0.33	0.10	0.30	0.15	0.10	0.26	0.31	-0.02
uNDF	-0.09	-0.03	0.13	0.19	0.05	-0.01	-0.22	-0.39+	-0.20	-0.26	0.10	0.18	0.22
Carbohydrate sub-fractions (%CHO)													
CA4	0.04	-0.03	0.19	0.13	0.12	0.06	-0.69**	-0.40+	-0.47*	-0.69**	0.23	0.42*	0.16
CB1	-0.18	-0.03	-0.03	-0.23	-0.17	-0.08	-0.17	-0.05	-0.16	-0.14	-0.12	0.06	0.11
CB2	0.02	-0.10	-0.16	0.06	0.12	0.02	0.27	0.05	0.11	0.28	-0.04	-0.13	0.04
CB3	0.30	0.23	0.16	0.18	-0.07	-0.01	-0.03	0.17	0.08	0.06	0.18	-0.09	-0.46*
CC	-0.09	-0.04	0.10	0.19	0.07	-0.02	-0.23	-0.38	-0.22	-0.26	0.11	0.19	0.23

Notes: STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; CHO: carbohydrates; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NSC: non-structural carbohydrate; uNDF: indigestible neutral detergent fiber at 288 h. based on CNCPS 6.5); CA4 = sugar (rapidly degradable carbohydrate fraction); CB1 = starch (intermediately degradable carbohydrate fraction); CB2 = soluble fiber (intermediately degradable carbohydrate fraction); CB3 = digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC = indigestible fiber (unavailable neutral detergent fiber); "+": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

Table 5.7. Correlation analyses between carbohydrate structure spectral characteristics and estimated energy profiles, rumen degradation parameters of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCHO:	CEC: TCH	CEC: STCH
Estimated energy profiles													
tdNFC (%DM)	0.04	-0.20	-0.12	0.04	0.19	0.15	0.35^{+}	0.09	0.18	0.27	-0.04	0.06	0.17
tdNDF (%DM)	0.12	0.28	0.09	0.05	-0.10	-0.11	0.11	0.03	0.20	0.09	-0.03	-0.14	-0.26
TDN1x (%DM)	-0.13	-0.15	-0.15	-0.20	-0.05	-0.05	-0.20	-0.32	-0.28	-0.19	-0.12	0.07	0.18
DE_{1x} (Mcal/kg)	-0.25	-0.19	-0.18	-0.27	-0.17	-0.15	-0.36^{+}	-0.31	-0.41^{+}	-0.30	-0.14	0.01	0.19
DEp_{3x} (Mcal/kg)	-0.23	-0.24	-0.19	-0.28	-0.19	-0.20	-0.34	-0.25	-0.39^{+}	-0.26	-0.14	-0.02	0.14
ME_dairy	-0.22	-0.14	-0.13	-0.20	-0.13	-0.12	-0.37^{+}	-0.34^{+}	-0.41*	-0.32	-0.11	0.05	0.20
NEl (Mcal/kg)	-0.17	-0.18	-0.14	-0.16	-0.12	-0.12	-0.34	-0.32	-0.39^{+}	-0.26	-0.08	0.05	0.17
ME_beef (Mcal/kg)	-0.25	-0.19	-0.20	-0.29	-0.17	-0.12	-0.30	-0.26	-0.34	-0.26	-0.18	-0.02	0.19
NE_m (Mcal/kg)	-0.24	-0.21	-0.20	-0.28	-0.20	-0.16	-0.34	-0.26	-0.40^{+}	-0.27	-0.16	-0.04	0.13
NE_g (Mcal/kg)	-0.22	-0.15	-0.13	-0.25	-0.15	-0.16	-0.34	-0.31	-0.38^{+}	-0.30	-0.14	0.02	0.18
Rumen degradation parameters													
RUNDF (g/kg DM)	0.35^{+}	0.20	0.23	0.44*	0.36^{+}	0.32	0.13	0.33	0.20	0.13	0.30	0.24	-0.01
EDNDF (g/kg DM)	0.03	0.05	0.00	-0.10	-0.28	-0.29	0.03	-0.11	-0.01	0.09	-0.10	-0.26	-0.36^{+}
BSt (g/kg DM)	0.07	-0.01	0.40^{+}	0.04	-0.17	-0.23	0.03	0.14	0.11	0.16	0.14	-0.09	-0.31
EDST (g/kg DM)	-0.08	-0.13	-0.17	-0.15	-0.02	0.07	-0.09	-0.09	-0.17	-0.10	-0.10	0.12	0.18
BCP (g/kg DM)	0.07	0.26	0.04	0.11	-0.16	-0.17	-0.04	0.16	-0.01	0.01	0.05	-0.14	-0.22
EDCP (g/kg DM)	-0.17	-0.17	0.01	-0.19	0.00	-0.01	-0.51*	-0.30	-0.48*	-0.39^{+}	0.04	0.18	0.16

Notes: STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; CHO: carbohydrates; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; tdNFC, truly digestible non-fiber carbohydrate; tdNDF: truly digestible neutral detergent fiber. TDN_{1x}: total digestible nutrient at one time maintenance. DE_{1x}: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; NE_{L3x}: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for growth. RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF: effective degraded neutral detergent fiber; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch; BCP: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; "$^{+}$": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

Table 5.8 Correlation analyses between carbohydrate structure spectral characteristics and intestinal digestion parameters, predicted truly absorbed protein supply of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

Item	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCH: TCHO	CEC: TCHO	CEC: STCHO
Intestinal digestion parameters													
TDNDF (g/kg DM)	0.04	-0.08	0.03	0.04	-0.22	-0.26	0.24	-0.02	0.13	0.36^{+}	-0.11	-0.27	-0.25
TDST (g/kg DM)	0.01	-0.17	0.12	-0.14	-0.10	-0.08	-0.02	0.04	-0.07	0.08	-0.03	0.05	-0.05
dIDP (%)	-0.35^{+}	-0.03	-0.04	-0.37^{+}	-0.25	-0.13	-0.04	-0.19	0.03	-0.16	-0.27	-0.13	0.13
IDP (g/kg DM)	-0.15	0.22	-0.02	-0.12	-0.40*	-0.33	-0.10	-0.02	-0.03	-0.06	-0.12	-0.29	-0.24
TDP (g/kg DM)	-0.32	0.00	-0.14	-0.25	-0.23	-0.17	-0.47*	-0.31	-0.42*	-0.40^{+}	-0.13	-0.02	0.13
DVE/OEB Model													
DVE (g/kg DM)	-0.18	0.17	-0.07	-0.11	-0.31	-0.22	-0.13	-0.03	-0.04	-0.18	-0.13	-0.20	-0.10
OEB (g/kg DM)	-0.16	-0.21	0.04	-0.19	-0.04	-0.10	-0.52*	-0.28	-0.49*	-	0.08	0.16	0.09
FMV (kg milk/kg	-0.15	0.17	-0.06	-0.09	-0.31	-0.24	-0.15	-0.02	-0.06	-0.18	-0.10	-0.18	-0.12
NRC Dairy Model													
RUP (g/kg DM)	0.07	0.26	0.04	0.11	-0.16	-0.17	-0.04	0.16	-0.01	0.01	0.05	-0.14	-0.22
MP (g/kg DM)	-0.19	0.21	-0.06	-0.15	-0.42*	-0.33	-0.08	-0.05	-0.02	-0.07	-0.17	-0.30	-0.19
DPB (g/kg DM)	-0.21	-0.13	0.00	-0.20	-0.03	-0.03	-0.51*	-0.32	-0.48*	-0.41^{+}	0.01	0.15	0.16
FMV (kg milk/kg	-0.16	0.21	-0.03	-0.14	-0.38^{+}	-0.31	-0.10	-0.03	-0.02	-0.09	-0.14	-0.28	-0.19

Notes: STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; TDNDF: total digested neutral detergent fiber; TDST: total digested starch; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value; "+": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method.

5.6.1.5. Multiple regression analysis between protein related molecular structure features and nutritional and metabolic characteristics of protein

A multiple regression analysis was performed in order to select the best variables to predict protein profiles, ruminal degradation kinetics, intestinal digestion and truly absorbed protein supply for dairy cattle. Variables with $P < 0.05$ were allowed to remain in the prediction equation, the percentage of the total variance represents the variance that could be explained by the model. Data from this study is presented in Table 5.9. Amide II area (A_AII) proved to be the best predictor for crude protein (CP, 60%) and truly digestible crude protein (tdCP, 60%).

5.6.1.6. Multiple regression analysis between carbohydrates (CHO) related molecular structure features and nutritional and metabolic characteristics of CHO

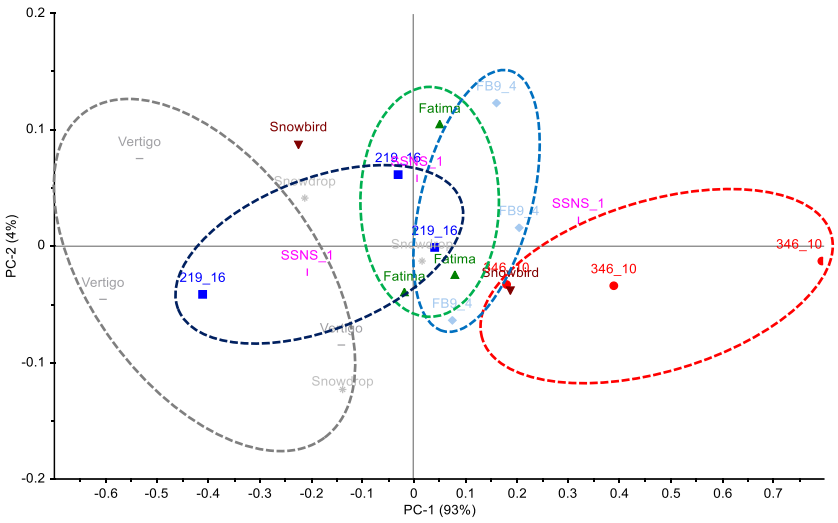
Table 5.10. shows the data for multiple regression between carbohydrates (CHO) related molecular structure features and nutritional and metabolic characteristics of CHO in different genotypes of faba bean grown in western Canada. The coefficients of variance (R^2) in this multiple regression analysis were very low (< 50%) to be consider as precise predictors of different metabolic characteristics.

5.6.1.7. Multivariate molecular spectral analysis for FTIR spectra

The principal component analysis (PCA) was able to group different genotypes of faba bean by its amide related region (Figure 5.4a). Principal component one (PC1) explained 93 % of the variation between spectra data related to the protein region. Vertigo was separated from 346_20, Fatima, and FB9_4 while 219_16 was grouped differently from 346_10. On the other hand, Snowdrop, Snowbird, 219_16, Fatima, and FB9_4 were overlapped among each other, implying a similar molecular structure in terms of protein make up. In terms of total carbohydrates (TCHO) (Figure 5.5a), PC1 explained the 85 % of variation between the CHO related molecular structure of

different genotypes of faba bean. Vertigo was also clearly separated from Fatima, FB9_4 and 346_10 while Snowdrop was differentiated from FB9_4 and 346_10. Fatima, FB9_4, 219_16, and 346_10 overlapped among each other.

a)



b)

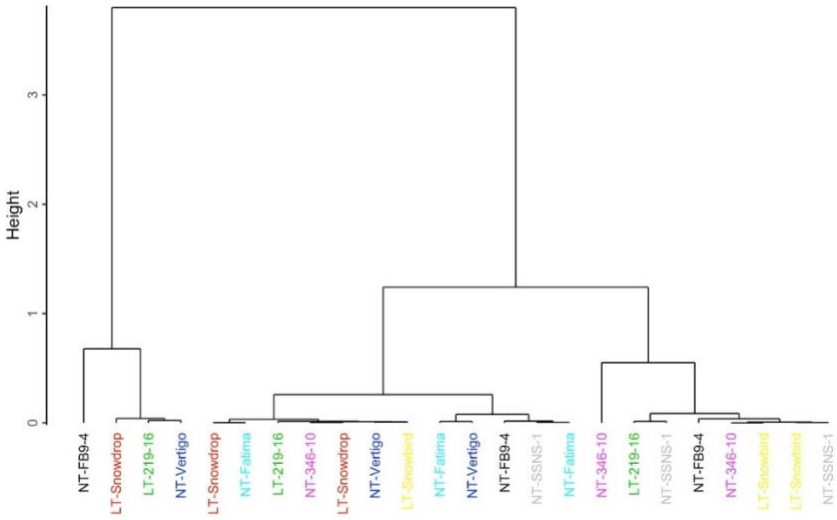
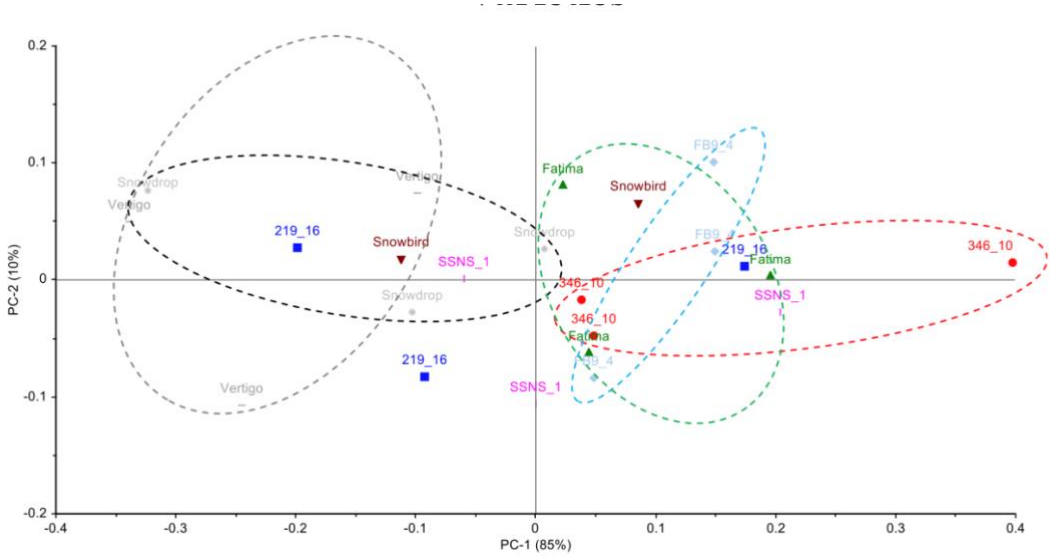


Figure 5.4. a) principal components analysis (PCA) and b) cluster analysis (CLA) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using FTIR vibrational at amide region (ca. 1718-1480 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2); CLA: Ward. D algorithm and Squared Euclidean distance. LT: low tannin; NT: normal tannin.

a)



b)

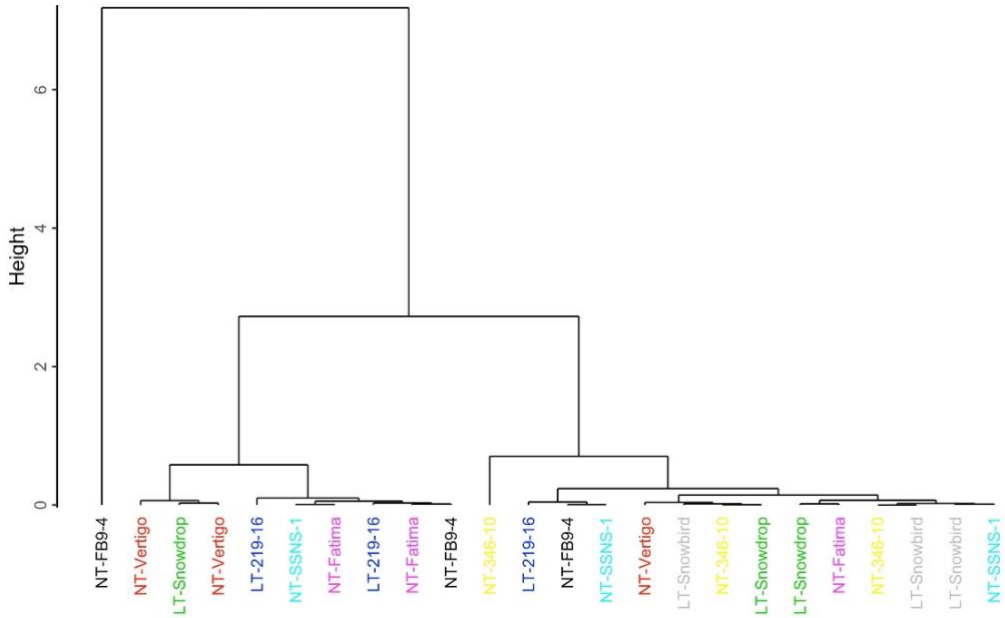


Figure 5.5. Principal components analysis (PCA) and cluster analysis (CLA) of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using FTIR vibrational at TCHO region (ca. 1185-942 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2); CLA: Ward. D algorithm and Squared Euclidean distance. LT: low tannin; NT: normal tannin.

Table 5.9. Multiple regression analysis to choose the most important protein spectral parameters for predicting protein profiles, estimated energy profiles, rumen protein degradation characteristics, intestinal digestion, and truly absorbed nutrient supply of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

predicted variable (y)	variable selection (P<0.05)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + ... + b_n \times x_n$	model R^2	RSD	P value
Protein profiles					
CP (% DM)	amide II area	CP = 11.35 + 1.48 × AII	0.60	0.79	<0.01
NDICP (% CP)	amide I to amide II area ratio	NDICP = 7.36 – 2.43 × A_AI_AII	0.23	0.36	0.02
NPN (% CP)	amide I area	NPN = -16.06 + 1.09 × AI	0.24	2.10	0.02
Protein sub-fractions					
PB2 (% CP)	amide I to amide II area ratio	PB2 = 23.27 – 7.39 × A_AI_AII	0.17	1.31	0.05
Estimated energy profiles					
tdCP (% DM)	amide II area	tdCP = 11.35 + 1.48 × AII	0.60	0.79	<0.01
DE_{1x} (Mcal/kg)	amide II area	DE1x = 3.66 + 0.03 × AII	0.29	0.03	0.01
DEp_{3x} (Mcal/kg)	amide II area	Dep3x = 3.33 + 0.03 × AII	0.33	0.02	<0.01
ME_beef (Mcal/kg)	amide II area	ME = 2.97 + 0.02 × AII	0.35	0.02	<0.01
NE_m(Mcal/kg)	amide II area	NEm = 2.04 + 0.02 × AII	0.30	0.02	0.01
NE_g(Mcal/kg)	amide II area	NEg = 1.39 + 0.01 × AII	0.26	0.01	0.01
Rumen protein degradation					
BCP (g/kg DM)	amide II area	BCP = -4.96 + 6.74 × AII	0.22	8.20	0.02
EDCP (g/kg DM)	total area amide I and amide II	EDCP = 76.48 + 2.51 × T_AI_AII	0.30	8.98	0.01
Intestinal protein digestion					
dIDP (%)	α-helix to β-sheet ratio	dIDP = -127.29 + 186.56 × H_α-helix: β-sheet	0.20	4.66	0.03
TDP (g/kg DM)	amide II area	TDP = 115.35 + 13.41 × AII	0.52	8.34	<0.01
DVE/OEB – NRC values					
OEB (g/kg DM)	total area amide I and amide II	OEB = -46.31 + 2.42 × T_AI_AII	0.29	8.81	0.01
DPB (g/kg DM)	α-helix peak height	DPB = -50.52 + 312.05 × α-helix	0.30	8.82	0.01

Notes: H_: peak height; AI: amide I area; AII: amide II area; A_AI_AII: amide I to amide II area ratio; T_AI_AII: total area amide I and amide II; H_α-helix:β-sheet: α-helix to β-sheet height ratio; CP: crude protein; NDICP: neutral detergent insoluble crude protein; SCP: soluble crude protein; NPN: non-protein nitrogen; PB2: slowly degradable protein; BCP: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; DPB: rumen degraded protein balance; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

Table 5.10. Multiple regression analysis to choose the most important CHO spectral parameters for predicting CHO profiles, estimated energy profiles, rumen degradation characteristics, intestinal digestion, and truly absorbed nutrient supply of different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada.

predicted variable (y)	variable selection (P<0.05)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + ... + b_n \times x_n$	model R^2	RSD	P value
Carbohydrate profiles					
CHO (% DM)	total CHO area	CHO = 51.56 + 0.24 × TCHO	0.17	1.13	0.045
ADF (% DM)	3rd peak structural CHO	ADF = 4.11 + 52.06 × H_1390	0.32	0.40	<0.01
cellulose (%DM)	3rd peak structural CHO	cellulose = 5.30 + 40.20 × H_1390	0.25	0.37	0.01
sugar (% DM)	3rd peak total CHO	sugar = 11.27 – 13.02 × H_1015	0.22	0.49	0.02
Carbohydrate sub-fractions					
CA4 (% CHO)	total CHO area	CA4 = 20.06 – 0.20 × TCHO	0.25	0.74	0.01
CB3 (% CHO)	cellulosic compounds to structural	CB3 = 40.65 – 103.30 × CEC: STCHO	0.22	1.44	0.02
Rumen CHO degradation					
RUNDF (g/kg DM)	3rd peak structural CHO	RUNDF = -1.10 + 811.53 × H_1390	0.22	8.10	0.02
EDCP (g/kg DM)	2nd peak total CHO	EDCP = 364.95 – 362.07 × H_1076	0.20	9.62	0.03
Intestinal CHO digestion					
TDP (g/kg DM)	total CHO area	TDP = 445.38 – 2.59 × TCHO	0.21	10.69	0.02
DVE/OEB – NRC values					
OEB (g/kg DM)	2nd peak total CHO	OEB = 235.20 – 356.77 × H_1076	0.20	9.37	0.03
DPB (g/kg DM)	total CHO area	DPB = 246.87 – 2.17 × TCHO	0.20	9.46	0.03

Notes: H_: peak height; TCHO: total CHO area; CEC: cellulosic compounds; STCHO: structural CHO; CHO: carbohydrates; ADF: acid detergent fiber; CA4 = sugar (rapidly degradable carbohydrate fraction; CB3 = digestible fiber (slowly degradable carbohydrate fraction); RUNDF: rumen bypass or undegraded feed neutral detergent fiber EDCP: effectively degraded crude protein; TDP: total digested protein; OEB: degraded protein balance; DPB: rumen degraded protein balance; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

5.6.2. Fourier transform infrared spectroscopy (FTIR) analysis of heat processing induced molecular structure changes in raw and heat treated faba bean with low and normal tannin levels

5.6.2.1. Univariate analysis of protein related molecular structure spectral profiles

The protein inherent structure can be changed with heat processing by modifying the noncovalent bonds within the molecules; heat induced changes in the primary and secondary protein structure have been previously revealed with FTIR (Yan et al. 2017).

A univariate analysis with spectra data was performed in order to describe the effects of heat treatments on the molecular structure of faba bean with low and normal tannin level (Figure 5.5). In the present study, interaction between tannin level and heat treatment was observed in different spectral features, detailed information is presented in Table 5.11. Steam Pressure (SP) proved to have a greater effect on the protein molecular structure of low tannin genotypes (LT) compared to normal tannin genotypes (NT). Higher absorbance was observed in LT with SP in the following parameters: total amide (61.65 AU), amide I area (42.57 AU), amide II area (19.08 AU), amide I peak height (0.506 AU), and amide II peak height (0.311 AU). On the other hand, Microwave Irradiation reduced the values of total amide, amide I, amide II areas, and amide I, amide II peak heights compared to SP; however, these results were not different from the Control (raw seeds). In normal tannin genotypes (NT), all three treatments had similar absorbance on the amide related spectra areas and peak heights. For the protein secondary structure parameters, SP has a larger effect on α-helix peak height in LT compared to the Control and MI; in NT the effect of thermal treatments was similar. In the case of β-sheet peak height, in LT only SP increased the value compared to the Control but in NT both SP and MI increased the absorbance of β-sheet compared to the raw sample. The area ratio of amide I to amide II was higher with MI in LT while in NT

both heat treatments increased this ratio. The opposite was observed in the ratio of α-helix to β-sheet peak height in which absorbance was decreased with SP and MI in both LT and NT. Similar outcomes have been previously published by Yu (2011) in which the author found a lower α-helix: β-sheet, higher amide I: amide II with moisture thermal treatment in soybean seeds. Yu (2005d) found that dry heating reduced the α-helix level and α-helix: β-sheet while increasing the β-sheet level in flaxseeds. Yan et al. (2014) reported changes in the protein molecular structure of barley grains with Microwave Irradiation which showed to be similar to some parameters found in this study. The difference in protein availability and digestibility is strongly related to the alterations of the complete protein structure. Heat induced changes in the secondary components of proteins could modify the degradability and digestibility of feed protein in barley grain resulting in decreased degradation in the rumen and higher supply of rumen undegraded protein to the small intestine of dairy cattle (Yan et al. 2014). Increased levels of β-sheet could decrease the access of digestive enzymes to proteins; the increase of β-sheet height after heating is due to the protein aggregations that are forming secondary intermolecular structures. Lower levels of α-helix to β-sheet ratios have been related to a larger protein fermentation in the rumen and lower digestion in the small intestine in pelleted samples (Huang, et al. 2017). Theodoridou and Yu (2013) stated that the denaturation of proteins during thermal treatments could be the cause of the change in α-helix: β-sheet ratio.

Table 5.11. Protein molecular structure spectral profiles of raw and heat treated faba bean grown in western Canada using Fourier transform infrared attenuated total reflectance molecular spectroscopy (FTIR).

Item		Low tannin genotypes			Normal tannin genotypes			SEM	P value		
		Control	Steam Pressure	Microwave Irradiation	Control	Steam Pressure	Microwave Irradiation		Tannin level (T)	Heat treatment (Heat)	T x Heat
	peak region and center (cm^{-1})	(n= 3)	(n= 3)	(n= 3)	(n= 3)	(n= 3)	(n= 3)				
Protein related primary structure											
total amide area	ca. 1480-1718	55.12^{bc}	61.65^{a}	51.82^{c}	56.6^{bc}	57.06^{ab}	59.12^{ab}	1.211	0.16	<0.01	<0.01
amide I area		38.18^{bc}	42.57^{a}	36.17^{c}	38.46^{bc}	39.83^{ab}	41.22^{ab}	0.803	0.19	<0.01	<0.01
amide II area		16.94^{bc}	19.08^{a}	15.65^{c}	18.14^{ab}	17.23^{abc}	17.90^{ab}	0.487	0.18	0.02	<0.01
amide I peak height	ca. 1640	0.471^{ab}	0.506^{a}	0.458^{b}	0.486^{ab}	0.494^{ab}	0.505^{a}	0.0091	0.03	0.04	0.01
amide II peak height	ca. 1534	0.295^{ab}	0.311^{a}	0.279^{b}	0.311^{a}	0.299^{ab}	0.307^{ab}	0.0071	0.06	0.21	0.02
Protein related secondary structure											
α-helix peak height	ca. 1647	0.461^{bc}	0.503^{a}	0.452^{c}	0.483^{abc}	0.477^{abc}	0.492^{ab}	0.0094	0.13	0.09	<0.01
β-sheet peak height	ca. 1627	0.409^{ab}	0.451^{a}	0.405^{ab}	0.387^{b}	0.450^{a}	0.451^{a}	0.0117	0.41	<0.01	0.01
Ratios											
amide I: II area		2.27^{ab}	2.24^{ab}	2.33^{a}	2.13^{b}	2.32^{a}	2.32^{a}	0.041	0.55	0.01	0.03
amide I: II height		1.60	1.63	1.65	1.57	1.66	1.65	0.018	0.74	<0.01	0.28
α-helix: β-sheet height		1.15^{ab}	1.12^{b}	1.12^{b}	1.27^{a}	1.06^{b}	1.09^{b}	0.03	0.56	<0.01	0.01

Notes: $^{a-c}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. The peak area and the peak height presented in each functional group measurements were expressed in IR absorbance units. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

5.6.2.2. Univariate analysis of carbohydrates and cellulosic compounds related molecular structure spectral profiles

Molecular structure spectral profiles related to carbohydrates (CHO) are presented in Table 5.12. The structural carbohydrates (STCHO) area was decreased only in LT with the SP treatment. Peak 1 of STCHO related to lignin spectra was not significantly different between treatments ($P > 0.10$). Peak 3 of STCHO increased with MI and showed the lowest absorbance with SP in LT, while in NT both SP and MI remained the same as the Control treatment. Cellulosic compounds (CEC) area and peak height was increased with SP while MI did not cause any changes compared to the Control in LT; the effect of SP in NT was lower than in LT but similar to the result of MI. In the case of the spectral profiles related to total carbohydrates (TCHO), MI in LT had a larger impact on the TCHO area, 1st, 2nd, and 3rd peak heights compared to SP and only in the 2nd peak absorbance was different from the Control. The effects of SP and MI were not different between each other in in TCHO spectral features of NT.

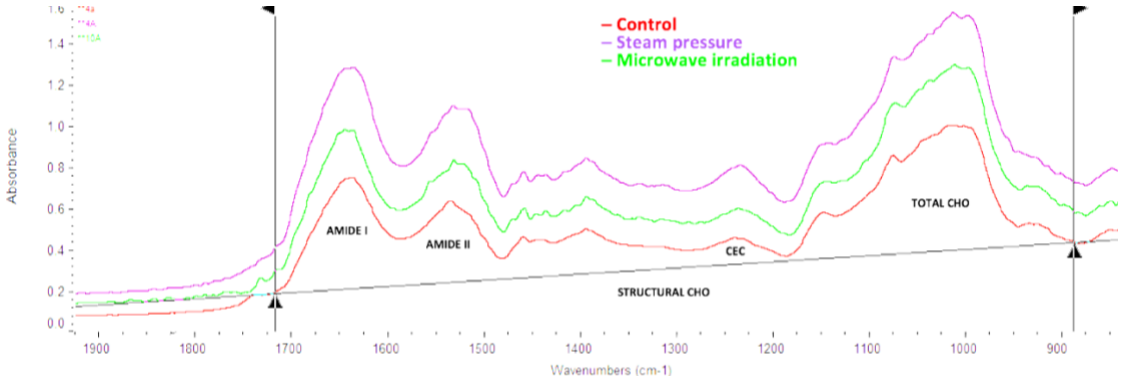


Figure 5.6. FTIR spectrum of the effect of heat processing treatments (Raw, Steam Pressure, and Microwave Irradiation) on different genotypes of faba bean with low and normal tannin level grown in western Canada at protein amide I and amide II related functional group region (ca. 1718-1480 cm^{-1}) and carbohydrate related functional group region (ca. 1481-942 cm^{-1}). Molecular structure of heat-treated normal tannin faba bean (Fatima).

Table 5.12. Carbohydrate molecular structure spectral profiles of raw and heat treated faba bean grown in western Canada using Fourier transform infrared attenuated total reflectance molecular spectroscopy (FTIR).

Item		Low tannin genotypes			Normal tannin genotypes			SEM	P value		
		Control	Steam Pressure	Microwave Irradiation	Control	Steam Pressure	Microwave Irradiation		Tannin level (T)	Heat treatment (Heat)	T x Heat
	peak region and center (cm^{-1})	(n= 3)	(n= 3)	(n= 3)	(n= 3)	(n= 3)	(n= 3)				
Structural CHO (STCHO) related to spectral profile											
1st peak height	ca.1518	0.028	0.033	0.031	0.033	0.027	0.033	0.0038	0.95	0.89	0.31
2nd peak height	ca. 1445	0.077	0.064	0.071	0.077	0.075	0.078	0.0026	0.01	0.02	0.10
3rd peak height	ca. 1390	0.125^{ab}	0.118^{b}	0.130^{a}	0.130^{a}	0.132^{a}	0.128^{a}	0.0022	<0.01	0.22	<0.01
STCHO area	ca. 1481 - 1185	19.26^{a}	17.00^{b}	18.62^{a}	19.09^{a}	18.81^{a}	19.47^{a}	0.386	0.01	<0.01	0.04
Cellulosic compounds (CEC) related to spectral profile											
peak height	ca. 1235	0.075^{b}	0.090^{a}	0.075^{b}	0.076^{b}	0.079^{b}	0.080^{b}	0.0018	0.29	<0.01	<0.01
CEC area	ca. 1185 - 942	3.59^{b}	4.25^{a}	3.69^{b}	3.66^{b}	3.75^{b}	3.89^{ab}	0.089	0.26	<0.01	<0.01
Total CHO (TCHO) related to spectral profile											
1st peak height	ca. 1145	0.181^{a}	0.162^{b}	0.183^{a}	0.173^{ab}	0.183^{a}	0.179^{a}	0.0035	0.35	0.06	<0.01
2nd peak height	ca. 1076	0.401^{b}	0.375^{c}	0.422^{a}	0.403^{ab}	0.407^{ab}	0.407^{ab}	0.0050	0.10	<0.01	<0.01
3rd peak height	ca. 1015	0.536^{ab}	0.472^{c}	0.559^{a}	0.515^{b}	0.542^{ab}	0.528^{ab}	0.0094	0.43	<0.01	<0.01
TCHO area	ca. 1185-942	67.69^{ab}	61.85^{c}	70.58^{a}	66.22^{b}	68.86^{ab}	68.09^{ab}	1.011	0.22	<0.01	<0.01
Area ratio related to spectral profile											
STCHO: TCHO		0.285^{a}	0.276^{ab}	0.264^{b}	0.289^{a}	0.273^{ab}	0.286^{a}	0.0046	0.04	0.01	0.03
CEC: TCHO		0.053^{b}	0.069^{a}	0.052^{b}	0.055^{b}	0.054^{b}	0.058^{b}	0.0020	0.21	<0.01	<0.01
CEC: STCHO		0.189^{b}	0.250^{a}	0.198^{b}	0.192^{b}	0.200^{b}	0.204^{b}	0.0065	0.01	<0.01	<0.01

Notes: a-c Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. STCHO (peak area region and baseline, ca. 1481 - 1185 cm−1); TCHO (peak area region and baseline, ca. 1185 - 942 cm−1); CEC (peak area region and baseline, ca. 1288−1185 cm−1). The peak area and the peak height presented in each functional group measurements were expressed in IR absorbance units. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1.

5.6.2.3. Correlation analysis between protein related molecular structure features with protein profiles and metabolic characteristics

The correlation *between* protein structure spectral characteristics and protein profiles are presented in Table 5.13. Acid detergent insoluble crude protein (ADICP) showed a positive correlation with β-sheet peak height (r = 0.48) and also tended to be correlated with total amide and amide I areas ($P < 0.10$). The protein sub-fractions showed a positive correlation between the undegradable protein fraction (PC) and β-sheet (r = 0.49). A negative association between true protein (TP) β-sheet (r = - 0.49) was observed while tended to be correlated with amide I area (r = -0.48), total amide area (r = -0.45), and amide I peak height (r = - 0.41). Tendencies were observed among some parameters: soluble crude protein (SCP) with amide I area (r = - 0.44), amide II area (r = - 0.45), and total amide area (r = - 0.47); rapidly degradable protein fraction (PA2) with amide II area (r = - 0.42), moderately degradable protein fraction (PB2) with amide I area and total amide area (r = 0.44 for both).

Correlation analysis results of protein structure molecular spectral characteristics and estimated energy values and rumen degradation kinetics are shown in Table 5.14. All energy profiles were significantly correlated with amide I to amide II peak height ($P < 0.05$): total digestible nutrients (TDN_{1x}) (r = - 0.56), digestible energy at 1x maintenance (DE_{1x}) (r = - 0.61), digestible energy of production at 3x maintenance (DEp_{3x}) (r = - 0.59), metabolizable energy (ME) based on NRC dairy and NRC beef (r = - 0.60 and r = - 0.57, respectively), net energy of lactation (NE_L) (r = - 0.59), net energy of maintenance (NE_m) (r = - 0.59), and net energy of gain (NE_g) (r = - 0.60). Furthermore, DE_{1x}, DEp_{3x}, ME dairy and beef, NE_L, and NE_g tended to have a negative correlation with β-sheet peak height ($P < 0.10$). Rumen degradation kinetics of protein did not show any association with protein related spectral features. The correlation study between the molecular

structure related to protein and the intestinal digestion and the truly absorbed nutrient supply parameters showed no significant correlation for either raw or heat treated faba bean. Complete data is presented in Table 5.15. The results from this study were not in full agreement with data published by Yan et al. (2017) where a positive correlation was observed between molecular structure features of protein with the total truly absorbable protein supply to dairy cattle (DVE), but no correlation was detected with the degraded protein balance (OEB). Carbonaro et al. (2012), found that dry heating and autoclaving produced structural changes in different legumes (white common beans, chickpea, lentil and soybean), detecting a higher effect on the secondary structure of proteins with autoclave heating; furthermore, they observed a higher content of β-sheet structures in legumes compared to other feed proteins which was associated with a lower protein digestibility. Thermal processing methods, specifically autoclaving has proven to decrease the β-sheet structures, improving the further digestibility of feed protein (Carbonaro et al. 2012).

Several studies have shown that rumen degradation kinetics and intestinal digestibility of feeds could be considerably affected by molecular structure changes, demonstrating FTIR could be used as a novel approach to predict nutrient utilization dairy cattle.

Table 5.13. Correlation analyses between protein structure spectral characteristics and protein profiles, protein sub-fractions of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Basic protein profiles										
CP (% DM)	0.06	0.10	-0.26	0.07	-0.02	0.03	-0.09	-0.02	0.07	-0.03
NDICP (% CP)	-0.20	0.11	-0.24	-0.16	-0.11	0.09	-0.19	0.07	-0.29	0.35
ADICP (% CP)	0.46^{+}	0.19	0.10	0.44^{+}	0.39	0.10	0.33	0.34	0.48*	-0.13
SCP (% CP)	-0.44^{+}	-0.45^{+}	0.30	-0.47^{+}	-0.34	-0.11	-0.18	-0.30	-0.35	-0.01
Protein sub-fractions										
PA2 (% CP)	-0.34	-0.42^{+}	0.34	-0.39	-0.24	-0.06	-0.15	-0.22	-0.26	-0.01
PB1 (% CP)	0.44^{+}	0.38	-0.26	0.44^{+}	0.30	0.10	0.11	0.23	0.36	-0.08
PB2 (% CP)	-0.22	0.08	-0.21	-0.19	-0.11	0.13	-0.27	0.04	-0.30	0.32
PC (% CP)	0.48^{+}	0.22	0.09	0.45^{+}	0.41^{+}	0.10	0.33	0.36	0.49*	-0.12
TP (% CP)	-0.48^{+}	-0.22	-0.09	-0.45^{+}	-0.41^{+}	-0.10	-0.33	-0.36	-0.49*	0.12
PA2tp (% CP)	-0.38	-0.42^{+}	0.34	-0.41^{+}	-0.28	-0.12	-0.11	-0.25	-0.30	0.00
PB1tp (% CP)	0.40	0.35	-0.23	0.40	0.27	0.05	0.17	0.20	0.34	-0.09
PB2tp (% CP)	-0.22	0.08	-0.21	-0.19	-0.11	0.13	-0.27	0.04	-0.30	0.32

Notes: DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; PA2 = rapidly degradable protein fraction; PB1 = moderately degradable protein fraction; PB2 = slowly degradable protein fraction; PC = unavailable protein fraction; tp = true protein; "$^{+}$": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

Table 5.14. Correlation analyses between protein structure spectral characteristics and estimated energy values, rumen protein degradation of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Truly digestible nutrients										
tdCP (%DM)	-0.02	0.06	-0.26	0.02	-0.08	0.02	-0.12	-0.07	-0.01	0.00
Estimated energy profiles										
TDN_{1x} (%DM)	-0.32	-0.14	-0.09	-0.32	-0.26	0.16	-0.56*	-0.14	-0.40	0.18
DE_{1x} (Mcal/kg)	-0.34	-0.13	-0.15	-0.33	-0.27	0.17	-0.61*	-0.14	-0.43^{+}	0.23
DEp_{3x} (Mcal/kg)	-0.36	-0.15	-0.13	-0.35	-0.30	0.14	-0.59*	-0.18	-0.43^{+}	0.20
ME_dairy	-0.37	-0.13	-0.16	-0.36	-0.31	0.13	-0.60*	-0.17	-0.47^{+}	0.26
NE_L (Mcal/kg)	-0.37	-0.14	-0.14	-0.35	-0.31	0.14	-0.59*	-0.17	-0.44^{+}	0.21
ME_beef (Mcal/kg)	-0.38	-0.16	-0.13	-0.37	-0.31	0.12	-0.57*	-0.19	-0.45^{+}	0.20
NE_m (Mcal/kg)	-0.32	-0.15	-0.11	-0.31	-0.25	0.18	-0.59*	-0.13	-0.39	0.19
NE_g (Mcal/kg)	-0.34	-0.13	-0.14	-0.34	-0.29	0.16	-0.60*	-0.15	-0.42^{+}	0.19
Rumen protein degradation										
BCP^{DVE} (g/kg DM)	0.24	0.28	-0.31	0.23	0.11	0.08	-0.06	0.08	0.16	-0.01
RUP^{NRC} (g/kg DM)	0.24	0.28	-0.31	0.23	0.11	0.08	-0.06	0.08	0.16	-0.01
EDCP (g/kg DM)	-0.31	-0.27	0.15	-0.29	-0.18	-0.03	-0.12	-0.12	-0.28	0.13

Notes: tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one time maintenance. DE1×: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NEm: net energy for maintenance; NEg: net energy for growth; BCP^{DVE}: rumen bypassed crude protein in DVE/OEB system; EDCP: effectively degraded crude protein; "$^{+}$": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

Table 5.15. Correlation analyses between protein structure spectral characteristics and intestinal protein digestion, predicted truly absorbed protein supply of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
Intestinal protein digestion										
dIDP (%)	0.09	0.00	0.07	0.08	-0.02	-0.14	0.31	-0.05	0.16	-0.18
IDP (g/kg DM)	0.30	0.28	-0.26	0.27	0.16	0.12	-0.06	0.13	0.24	-0.07
TDP (g/kg DM)	-0.04	0.01	-0.18	0.02	-0.14	-0.04	-0.05	-0.09	-0.04	0.03
DVE/OEB model										
DVE (g/kg DM)	0.29	0.28	-0.25	0.27	0.16	0.12	-0.03	0.11	0.24	-0.10
OEB (g/kg DM)	-0.28	-0.20	0.09	-0.25	-0.17	0.00	-0.19	-0.07	-0.29	0.20
FMV (kg milk/kg	0.28	0.26	-0.24	0.26	0.15	0.11	-0.02	0.09	0.24	-0.10
NRC values										
MP (g/kg DM)	0.25	0.23	-0.24	0.22	0.12	0.09	-0.04	0.09	0.21	-0.06
DPB (g/kg DM)	-0.32	-0.31	0.23	-0.30	-0.19	-0.12	0.00	-0.17	-0.26	0.05
FMV (kg milk/kg	0.27	0.26	-0.25	0.24	0.14	0.11	-0.05	0.11	0.22	-0.07

Notes: IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value in DVE/OEB system; MP: metabolizable protein; DPB: rumen degraded protein balance; "+": $P<0.10$, not significant, but with a tendency; "*": $P<0.05$, significant; "**": $P<0.01$, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

5.6.2.4. Correlation analysis between carbohydrates (CHO) related molecular structure features with CHO profiles and metabolic characteristics

Table 5.16. shows the results from the correlation analysis between the molecular structure related to CHO and CHO profiles and sub-fractions of raw and heat treated faba bean. The indigestible neutral detergent fiber ($uNDF_{288h}$) and the unavailable neutral detergent fiber fraction (CC) were positively correlated with the ratio of structural carbohydrates (STCHO) to total carbohydrates (TCHO) (r = 0.58 and 0.57, respectively). On the other hand, the same parameters were negatively correlated with cellulosic compounds (CEC) to STCHO ratio (r = - 0.50 and - 0.49, respectively). Some parameters tended to have correlations among them ($P < 0.10$), including: carbohydrates (CHO) with CEC peak height (r = - 0.45); acid detergent lignin (ADL) with CEC area and peak height (r = 0.43 and r = 0.45), respectively; sugar with STCHO to TCHO ratio (r = 0.42), $uNDF_{288h}$ with STCHO 2nd peak height; rapidly degradable CHO fraction (CA4) with the ratio of STCHO to TCHO; and finally, CC with the 2nd peak height of STCH.

The results related to association of CHO molecular structure, energy profiles, and rumen degradation characteristics are presented in Table 5.17. The truly digestible neutral detergent fiber (tdNDF) tended to have a positive correlation with the 3rd TCHO peak height while a negative tendency was found between the total digestible nutrients (TDN), digestible energy of production at 3x maintenance (DEp_{3x}), net energy of lactation (NE_L), net energy of maintenance (NE_m), and net energy of gain (NE_g) with the CEC area. In addition, a tendency ($P < 0.10$) for correlation was found between the rumen undegraded or bypass starch (BST) with the CEC peak height (r = 0.43) and the 2nd and 3rd peaks of TCHO (r = - 0.46 and r = - 0.43, respectively). A significant association ($P < 0.05$) was observed between the effective degradable starch (EDST) and the TCHO 2nd peak

height (r = - 0.54). On the other hand, the rumen undegraded protein was negatively correlated with the 2nd TCHO peak (r = - 0.48).

A negative correlation was observed between the total digested neutral detergent fiber (TDNDF) and the ratio of STCHO to TCHO (r = -0.48). The intestinal digestibility of protein (dIDP) was also negatively correlated with the 3rd peak of STCHO (r = - 0.54). Additionally, the intestinal digestible rumen bypass protein (IADP), the truly digested protein in the small intestine (DVE), the feed milk value (FMV), the rumen undegradable crude protein (RUP), and the metabolizable protein (MP) had negative correlation with the 2nd TCHO peak height (r = - 0.54, - 0.54, - 0.52, - 0.48, and – 0.50, respectively). Complete data of correlation analysis from CHO related molecular structure, intestinal digestibility parameters and the predicted truly absorbed nutrient supply is presented in Table 5.18.

5.6.2.5. Multiple regression analysis between protein and carbohydrates related molecular structure features and nutritional and metabolic characteristics of protein and carbohydrates.

This study showed that some nutritional and metabolic protein characteristics as well as the energy values could be predicted with protein related molecular structure features. The peak height ratio of amide I to amide II was the predictor left in the model to calculate all related energy parameters, including: total digestible nutrients (TDN_{1x}, 67 % of variance explained), digestible energy at 1x maintenance (DE_{1x}, 70 % of variance), digestible energy of production at 3x maintenance (DEp_{3x}, 71 % of variance), metabolizable energy (ME-dairy, 71 % of variance), net energy of lactation (NE_L, 72 % of variance), ME-beef (70 % of variance), net energy of maintenance (NE_m, 70 % of variance), and net energy of gain (NE_g) with 71 % of variance explained by the model. Detailed data of multiple regression analysis of protein and CHO molecular structure profiles and protein

and CHO nutritional and metabolic characteristics of raw and heat treated faba bean are presented in Table 5.19. Sun et al. (2018b) stated that the ratios of amide I to II and α-helix to β-sheet are better predictors of protein nutritive values and digestibility, the authors also denoted that the use of larger number of samples could further explain the actual correlation between molecular spectral features and several metabolic characteristics in dairy cattle. In general, multiple regression analyses are a valuable feature to predict nutrient values of different feeds which are required for the correct formulation of animal rations.

5.6.2.6. Multivariate molecular spectral analysis for FTIR spectra

Figure 5.7 shows that principal component analysis (PCA) distinguished between the effect of steam pressure (SP) in low tannin genotypes (LT) and normal tannin genotypes (NT) in the amide related region (ca. 1480-1718 cm^{-1}) in which the variability was explained by 96 % in the first principal component (PC1). However, principal component two (PC2) was able to separate the treatments into separate different groups.

Table 5.16. Correlation analyses between carbohydrate structure spectral characteristics and carbohydrate profiles, carbohydrate sub-fractions of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCH: TCHO	CEC: TCHO	CEC: STCHO
Basic nutrient profiles (%DM)													
CHO	0.09	0.03	0.23	-0.05	-0.33	-0.45^{+}	0.25	0.13	0.33	0.21	0.05	-0.25	-0.17
NDF	-0.06	0.17	-0.20	0.10	0.24	0.01	0.27	-0.02	0.34	0.27	-0.34	0.01	0.15
ADF	-0.28	0.14	-0.34	-0.22	0.17	0.01	0.15	-0.06	0.19	0.11	-0.40	0.07	0.31
ADL	-0.16	0.08	-0.36	0.06	0.43^{+}	0.45^{+}	-0.11	-0.19	-0.17	-0.19	-0.20	0.34	0.33
Hemicellulose	-0.02	0.14	-0.16	0.24	0.35	0.24	0.04	-0.09	0.08	0.13	-0.14	0.14	0.13
Cellulose	-0.17	0.08	-0.16	-0.20	-0.04	-0.19	0.21	0.06	0.26	0.19	-0.25	-0.07	0.14
Starch	-0.12	0.01	-0.21	-0.09	0.26	0.21	0.12	0.03	0.09	0.06	-0.29	0.10	0.27
NFC	0.00	0.13	0.19	-0.02	-0.18	-0.13	-0.12	-0.08	-0.01	-0.10	0.18	-0.03	-0.05
Sugar	0.03	0.07	0.17	-0.06	-0.18	-0.10	-0.31	-0.16	-0.27	-0.22	0.42^{+}	-0.05	-0.21
NSC	-0.06	-0.03	-0.10	-0.10	0.15	0.12	0.03	0.03	0.03	0.04	-0.09	0.02	0.14
uNDF	0.36	-0.10	0.43^{+}	0.26	-0.38	-0.30	0.00	0.14	0.01	0.05	0.58*	-0.25	-0.50*
Carbohydrate sub-fractions (%CHO)													
CA4	0.03	0.05	0.17	-0.06	-0.15	-0.07	-0.33	-0.16	-0.29	-0.24	0.46^{+}	-0.02	-0.21
CB1	-0.13	-0.07	-0.24	-0.12	0.25	0.24	0.10	0.08	0.01	0.07	-0.31	0.09	0.26
CB2	-0.04	0.16	0.16	-0.11	-0.24	-0.13	-0.25	-0.17	-0.16	-0.24	0.20	-0.01	-0.09
CB3	-0.13	-0.10	-0.33	-0.17	0.13	0.01	0.18	0.06	0.05	0.15	-0.36	0.00	0.14
CC	0.35	-0.10	0.41^{+}	0.27	-0.37	-0.29	0.00	0.14	0.00	0.05	0.57*	-0.25	-0.49*

Notes: STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; CHO: carbohydrates; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; ; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NSC: non-structural carbohydrate; uNDF: indigestible neutral detergent fiber at 288 h based on CNCPS 6.5); CA4 = rapidly degradable carbohydrate fraction; CB1 = intermediately degradable carbohydrate fraction; CB2 = intermediately degradable carbohydrate fraction; CB3 = slowly degradable carbohydrate fraction; CC = unavailable neutral detergent fiber; "$^{+}$": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

Table 5.17. Correlation analyses between carbohydrate structure spectral characteristics and estimated energy profiles, rumen degradation parameters of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCH: TCHO	CEC: TCHO	CEC: STCHO
Estimated energy profiles													
tdNFC (% DM)	0.00	0.13	0.19	-0.02	-0.18	-0.13	-0.12	-0.08	-0.01	-0.10	0.18	-0.03	-0.05
tdNDF (% DM)	0.14	-0.17	0.02	0.17	-0.13	-0.28	0.36	0.25	0.32	0.43^{+}	-0.09	-0.24	-0.20
TDN_{1x} (% DM)	0.13	0.00	0.35	0.01	-0.40^{+}	-0.32	-0.05	0.06	0.03	0.02	0.24	-0.21	-0.26
DE_{1x} (Mcal/kg)	0.14	0.05	0.36	0.04	-0.39	-0.31	-0.09	0.00	0.01	-0.04	0.26	-0.19	-0.26
DEp_{3x} (Mcal/kg)	0.15	0.01	0.36	0.04	-0.41^{+}	-0.33	-0.08	0.03	0.02	-0.02	0.25	-0.21	-0.27
ME-dairy (Mcal/kg)	0.12	0.08	0.33	0.03	-0.38	-0.32	-0.08	-0.01	0.03	-0.02	0.25	-0.19	-0.25
NE_L(Mcal/kg)	0.14	0.02	0.35	0.06	-0.41^{+}	-0.33	-0.06	0.04	0.05	0.00	0.24	-0.22	-0.27
ME-beef (Mcal/kg)	0.13	0.02	0.33	0.04	-0.40	-0.32	-0.07	0.03	0.03	-0.01	0.22	-0.20	-0.25
NE_m (Mcal/kg)	0.18	-0.01	0.38	0.07	-0.41^{+}	-0.33	-0.05	0.06	0.05	0.00	0.27	-0.22	-0.30
NE_g (Mcal/kg)	0.14	0.01	0.35	0.04	-0.41^{+}	-0.32	-0.07	0.04	0.03	-0.01	0.23	-0.20	-0.26
Rumen degradation parameters													
RUNDF (g/kg DM)	0.15	-0.01	0.13	0.28	0.28	0.32	0.02	0.05	0.09	0.04	0.01	0.19	0.11
EDNDF (g/kg DM)	-0.12	0.17	-0.27	-0.01	0.19	-0.05	0.26	-0.05	0.28	0.23	-0.35	-0.01	0.14
Bst (g/kg DM)	-0.25	0.19	-0.35	-0.25	0.38	0.43^{+}	-0.38	-0.35	-0.46^{+}	-0.43^{+}	-0.14	0.44^{+}	0.37
EDST (g/kg DM)	0.23	-0.13	0.29	0.16	-0.27	-0.40	0.45^{+}	0.36	0.54^{*}	0.46^{+}	0.06	-0.38	-0.24
BCP (g/kg DM)	-0.17	0.01	-0.21	-0.05	0.27	0.44^{+}	-0.38	-0.23	-0.48^{*}	-0.33	-0.06	0.34	0.21
EDCP (g/kg DM)	0.16	0.08	0.25	0.12	-0.31	-0.40	0.23	0.10	0.37	0.19	0.09	-0.30	-0.23

Notes: H_: peak height; STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; CHO: carbohydrates; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; tdNFC, truly digestible non-fiber carbohydrate; tdNDF: truly digestible neutral detergent fiber. $TDN_{1\times}$: total digestible nutrient at one time maintenance. $DE_{1\times}$: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for growth. RUNDF: rumen bypass fiber; EDNDF: effective degraded neutral detergent fiber; BSt: rumen bypass starch; EDST: effective degraded starch; BCP: rumen bypassed crude; EDCP: effectively degraded crude protein; TDNDF: total digested neutral detergent fiber; "+": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

Table 5.18. Correlation analyses between carbohydrate structure spectral characteristics and intestinal digestion parameters, predicted truly absorbed protein supply of raw and heat treated faba bean seeds with low and normal tannin levels grown in western Canada.

	Structural Carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total Carbohydrates (TCHO)				Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	area	peak	area	1st peak	2nd peak	3rd peak	STCH: TCHO	CEC: TCHO	CEC: STCHO
Intestinal digestion parameters													
TDNDF (g/kg DM)	-0.27	0.15	-0.43+	-0.04	0.27	0.07	0.20	-0.07	0.24	0.23	-0.48*	0.05	0.29
TDST (g/kg DM)	-0.28	0.18	-0.33	-0.24	0.40	0.23	-0.06	-0.20	0.03	-0.10	-0.33	0.27	0.44+
dIDP (%)	-0.40	-0.11	-0.33	-0.54*	0.02	0.07	-0.15	-0.08	-0.32	-0.12	-0.45+	0.09	0.27
IDP (g/kg DM)	-0.25	-0.02	-0.24	-0.21	0.23	0.41+	-0.39	-0.23	-0.54*	-0.35	-0.15	0.35	0.27
TDP (g/kg DM)	-0.22	0.06	-0.21	-0.21	0.06	0.23	-0.33	-0.21	-0.40	-0.30	-0.23	0.18	0.20
DVE/OEB model													
DVE (g/kg DM)	-0.25	-0.06	-0.23	-0.20	0.22	0.41+	-0.39	-0.20	-0.54*	-0.33	-0.14	0.33	0.25
OEB (g/kg DM)	0.19	0.13	0.25	0.12	-0.26	-0.35	0.17	0.05	0.33	0.12	0.17	-0.23	-0.22
FMV (kg milk/kg feed)	-0.25	-0.07	-0.22	-0.20	0.21	0.39	-0.37	-0.19	-0.52*	-0.32	-0.15	0.31	0.24
NRC values													
RUP (g/kg DM)	-0.17	0.01	-0.21	-0.05	0.27	0.44+	-0.38	-0.23	-0.48*	-0.33	-0.06	0.34	0.21
MP (g/kg DM)	-0.23	-0.03	-0.23	-0.19	0.20	0.37	-0.36	-0.21	-0.50*	-0.32	-0.16	0.31	0.24
DPB (g/kg DM)	0.23	0.02	0.24	0.18	-0.27	-0.39	0.32	0.19	0.45+	0.30	0.14	-0.34	-0.27
FMV (kg milk/kg feed)	-0.24	-0.02	-0.23	-0.20	0.22	0.39	-0.38	-0.22	-0.52*	-0.34	-0.16	0.33	0.25

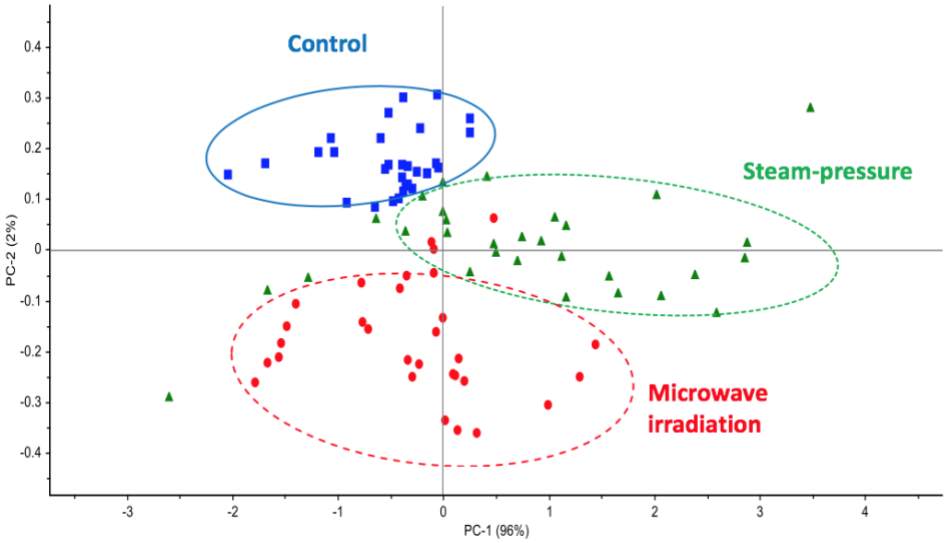
Notes: H_: peak height; STCHO1: H_1518; STCHO2: H_1445; STCHO3: H_1390; TCHO1: H_1145; TCHO2: H_1076; TCHO3: H_1015; CEC_H: 1235; CHO: carbohydrates; NFC: non-fiber carbohydrate; RD: rumen degradable; RU: rumen undegradable; ID: intestinal digestibility; TDNDF: total digested neutral detergent fiber; TDST: total digested starch; dIDP: intestinal digestibility of crude protein; IDP: intestinal digested protein; TDP: total digested protein; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value in DVE/OEB system; "+": P<0.10, not significant, but with a tendency; "*": P<0.05, significant; "**": P<0.01, strongly significant. r: correlation coefficient using spearman method. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

Table 5.19. Multiple regression analysis to choose the most important protein and CHO spectral parameters for predicting protein profiles, estimated energy profiles, intestinal carbohydrate digestion of raw and heat treated faba bean with low and normal tannin levels grown in western Canada.

predicted variable (y)	variable selection (P<0.05)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + ... + b_n \times x_n$	model R^2	RSD	P value
Protein profiles					
ADICP (%CP)	β-sheet peak height	ADICP = -0.70 + 2.34 × β-sheet	0.24	0.20	0.04
PC (%CP)	β-sheet peak height	PC = -2.42 + 8.21 × β-sheet	0.24	0.72	0.04
Estimated energy profiles					
TDN_{1x} (%DM)	amide I to amide II height	TDN_{1x} = 110.96 – 16.99 × H_AI_AII	0.67	1.01	0.01
DE_{1x} (Mcal/kg)	amide I to amide II height	DE_{1x} = 5.10 – 0.75 × H_AI_AII	0.70	0.04	0.01
DEp_{3x} (Mcal/kg)	amide I to amide II height	DEp_{3x} = 4.69 – 0.69 × H_AI_AII	0.71	0.04	0.01
ME_dairy (Mcal/kg)	amide I to amide II height	ME = 4.30 – 0.70 × H_AI_AII	0.71	0.04	0.01
NE_L (Mcal/kg)	amide I to amide II height	NE_L = 2.80 – 0.48 × H_AI_AII	0.72	0.02	0.01
ME_beef (Mcal/kg)	amide I to amide II height	ME = 4.16 – 0.60 × H_AI_AII	0.70	0.03	0.01
NE_m (Mcal/kg)	amide I to amide II height	NE_m = 3.03 – 0.52 × H_AI_AII	0.70	0.03	0.01
NE_g (Mcal/kg)	amide I to amide II height	NE_g = 2.19 – 0.42 × H_AI_AII	0.71	0.02	0.01
Intestinal CHO digestion					
TDNDF (g/kg DM)	structural CHO to total CHO	TDNDF = 370.80 - 947.38 × STCHO: TCHO	0.31	22.31	0.02

Notes: H_: peak height; H_AI_AII: amide I to amide II height ratio; TCHO: total CHO area; STCHO: structural CHO; STCHO: TCHO, structural CHO to total CHO ratio; ADICP: acid detergent insoluble crude protein; PC = unavailable protein fraction; $TDN_{1\times}$: total digestible nutrient at one time maintenance. $DE_{1\times}$: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for growth. RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level. Low tannin genotypes (n=3): Snowbird, Snowdrop, and 219_16; normal tannin genotypes (n=3): Fatima, 346_10, SSNS_1. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

a)



b)

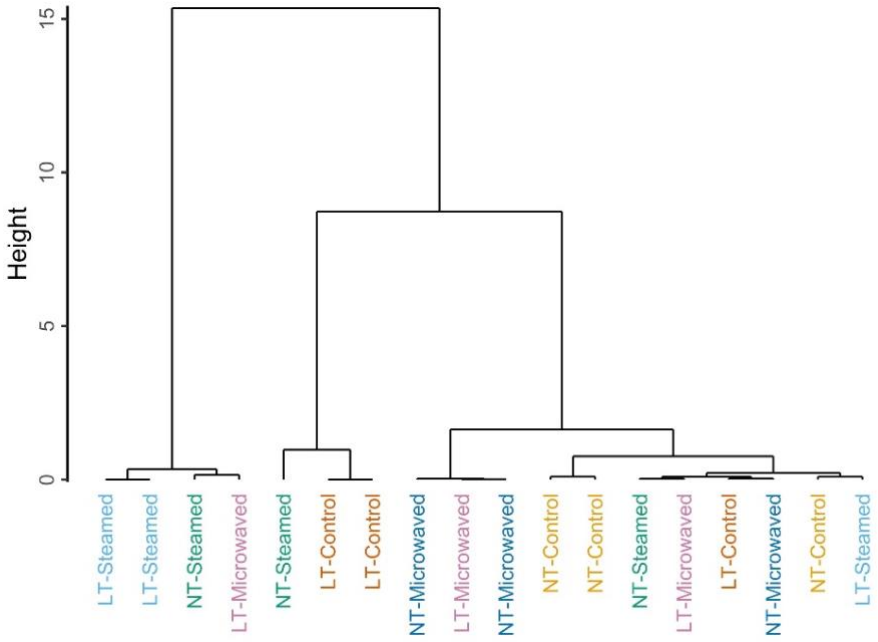


Figure 5.7. a) principal component analysis (PCA) and b) cluster analysis (CLA) of the effect of heat treatment in different genotypes of faba bean seeds with low and normal tannin levels grown in western Canada using FTIR vibrational at amide region (ca. 1718-1480 cm^{-1}). PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2); CLA: Ward. D algorithm and Squared Euclidean distance. LT: low tannin; NT: normal tannin. Heat processing methods (n= 3): Raw (Control), Steam Pressure, Microwave Irradiation).

5.7. Chapter summary and conclusions

Higher absorbance for amide I and amide II peak height and area, as well as for structural CHO (STCHO), total CHO (TCHO) area and peaks (H_1015, H_1076, H_1145), and cellulosic compounds (CEC) to total CHO (TCHO) ratio were found in normal tannin genotypes. Amide I area and α-helix absorbance were not different among low tannin genotypes; however, higher ratios for α-helix to β-sheet height and CEC to STCHO were observed. A positive correlation was found between the intensity absorbance (AU) of amide region, amide I, amide II areas and peak heights, α-helix, and β-sheet with CP, truly digestible crude protein (tdCP), effectively degraded crude protein (EDCP), total digested protein (TDP), and degraded protein balance (OEB and DPB). CHO were correlated positively with TCHO area and H_1076 peak while sugars were negatively associated with TCHO area, H_1076, and H_1145 peak heights, and CEC to TCHO ratio. TDP, OEB, and DPB were negatively related to TCHO and H_1076. Related to the heat induced changes in the molecular structure of faba bean, α-helix was increased with Steam Pressure in low tannin genotypes while β-sheet was higher than Control with Steam Pressure and Microwave Irradiation in normal tannin genotypes. Amide I to amide II ratio was increased with Steam Pressure and Microwave Irradiation in normal tannin genotypes, but α-helix to β-sheet ratio was reduced. Steam Pressure also increased the absorbance in amide I and amide II area while Microwave Irradiation was similar to the Control in low tannin genotypes. Furthermore, Steam Pressure increased the absorbance in the structural CHO (STCHO) area, cellulosic compounds (CEC) area and peak height (H_1235), CEC:TCHO ratio, and CEC:STCHO ratio while decreased the absorbance of all parameters related to total CHO (TCHO) spectral profile. On the other hand, Microwave Irradiation increased the absorbance of all parameters related to total CHO spectral profile, reduced the absorbance of STCHO:TCHO ratio, and showed similar results to Control in

the rest of parameters. Correlation among variables were found between ADICP, PC, and TP with β-sheet. All energy profiles were negatively correlated with Amide I to amide II ratio. The associations related to CHO spectral profiles were the following: dIDP with H_1390 (STCHO); EDST, BCP, DVE, RUP, MP and FMV with H_1076 (TCHO); uNDF with STCHO to TCHO and CEC to STCHO ratios; finally, TDNDF with STCHO to TCHO ratio. Significant correlations were found between protein and CHO spectral profiles and chemical and metabolic characteristics of faba bean which indicates that structural spectral features could be used to predict metabolic characteristics and nutrient availability in ruminants. This is important for further diet formulation, breeding selection programs, and prediction of quality and nutritive value of the feeds as this technique is less expensive, less time consuming, less invasive and requires a small amount of sample compared to other trials and chemical analyses. In the present study the molecular structure spectral characteristics related to protein and carbohydrates spectral features were different among genotypes and tannin levels of faba bean grown in western Canada. Additionally, FTIR analysis proved to be a useful tool to detect the responses of faba bean to heat processing methods by the different molecular structure changes induced by the treatments.

6. RESEARCH DISCUSSION AND CONCLUSION

Specific data related to condensed tannin analysis in faba bean is hard to compare, as different analytical methods could be used to determine phenolic compounds. Published data by Makkar et al. (1997) was the most similar to this research. They found a CT content of 2.6 % in colored flowered faba bean while in white colored flowers, CT (as leucocyanidin equivalent) could not be detected. Condensed tannins are capable of precipitating proteins and form soluble or insoluble complexes with various molecules (Addisu, 2016; Acuña et al. 2008; Frutos et al. 2004). Protein-tannin complexes are generally unstable as the bonds continually break and re-form. The hydrogen bonds are stable at pH of 3.5 and about 8, but when values go above or below this point the bonds tend to breakdown (Bunglavan and Dutta, 2013). The protein utilization efficiency can be increased by condensed tannins which can protect the protein from fast degradation in the rumen. Moderate amounts of CT (< 5 % of DM) can increase the amount of protein flow to the small intestine which are favorable if a higher absorption is also induced. A lower digestibility of protein in tannin containing feeds could be associated with persisting tannin-protein complexes, complexes formed with endogenous proteins or enzymes, tannin binding with proteins in the small intestine or tannin interaction with the intestinal mucosa (Theodoridou, 2010).

In the present study, the range of CP content was between 26.8 to 29.5 % DM, which was similar to results reported by Frejnagel et al. (1997) who obtained values around 27.2 to 28.9 % DM. The total carbohydrates of faba bean grown in western Canada used in the present study, showed variability in their content among genotypes. Bhatty (1973) found that proteins and carbohydrates were the most variable fractions among 12 different faba bean cultivars and proved to be inversely related to each other. Faba bean carbohydrates include water soluble starch, water soluble polysaccharides, water insoluble starch, free sugars, cellulose, hemicellulose and lignin (Bhatty,

1973). These inherent factors along with different methods of analysis could contribute to the final variation in CHO profiles. Faba bean has a high starch content as the principal carbohydrate storage in the seed of most legumes is represented by this nutrient, accounting for 22 to 45 % depending on the type of feed (Hoover and Sosulski, 1991). Some of the starch can bypass the rumen and undergo digestion later in the small intestine, so for that reason faba bean energy values are partly as good as cereal grains such as barley.

Regarding feed protein degradation, one important objective of ruminant nutrition is to reach the optimal microbial synthesis with less nitrogen loss in the rumen to provide an adequate protein supply to dairy cows. Tamminga et al. (1994, 2007) and Sinclair et al. (1993) imply that the ideal proportion of effective degradable N to energy is 25 g of available N per kg of available OM or 32 g of available N per kg of available CHO. When these values are lower it could implicate a shortage of N (source for microbial growth); conversely, when they increase, this could indicate a potential N loss from the rumen with less energy available to rumen microbes (Nuez-Ortín and Yu, 2010). Optimizing nutrient utilization is essential in the dairy industry.

Processing methods are used to change the physical, molecular and nutritional characteristics of different feedstuffs with the purpose of making feed ingredients more digestible and available to ruminants. In legumes, heat treatment can remove or inhibit antinutritional factors and improve the quality of the seeds. However, heat can affect feed nutrients in diverse forms. Altering the protein degradative behaviour with heat involves primarily denaturation of the protein secondary structure, this process relates to the organization of the general molecular structure of proteins by breaking hydrogen bridges and disulfide bonds (Salazar-Villanea et al. 2016). Heating methods result in protein structure stabilization and cross-linkages to carbohydrates. This effect protects proteins from ruminal hydrolysis, reducing their solubility and degradation rate in the rumen

(Goelema et al. 1999). During the heating process, Maillard reactions can occur, causing microbial resistance of proteins to proteolysis. These reactions are reversible in early stages when the temperature is moderated. In this case, rumen degradability of protein can be reduced without negative effects on digestibility (Andrade-Montemayor et al. 2009). Results related to the heat processing study, agreed with data published by Mustafa et al. (1998), where the rapidly degradable fraction was decreased and the intermediately and slowly degradable fractions were increased after autoclaving. The effects of SP and MI also agreed with previous published data by Yu (2005b) which observed that pressure toasting decreased the S fraction and increased D fraction. In the same study the Kd value was decreased as well but this disagreed with the results of the present MI effect. The effects of heat treatments on the metabolic characteristics of faba beas grown in western Canada, agreed with Yu et al. (2004, 2005b) in which the DVE and MP values were higher due to a significant increase in the truly absorbed bypass protein which could compensate the lower microbial protein digested in the small intestine. Heat treatments in this study did not sufficiently reduced the OEB and DPB values in faba bean as levels were considered high enough to cause a potentially N loss in the rumen (Yu et al. 2004, 2005b).

In terms of molecular features, infrared spectroscopy can be applied to reveal the inherent molecular structure in a wide range of elements. The molecular structure of feed nutrients is an important factor to understand the metabolic characteristics in feed analysis. FTIR can be used to study the biomolecular spectral features of feeds due to gene alteration or heat processing changes without the damage related to the reagents used in common chemical analysis. The molecular characteristics of feeds and their inherent structure are highly related with feed quality, nutritive value, and nutrient utilization (Yang and Yu, 2016; Yu, 2005c; Stuart, 2004). Carbonaro et al. (2014) stated that a large β-sheet and low α-helix component characterize the protein structure of

legume seeds and other plant proteins such as cereal grains. Wang et al. (2013) also specified that the nutrient difference between white and green broad beans is possibly caused by the difference in their secondary protein structure. This is important as previous studies have shown a negative correlation between the content of β-sheet and protein digestibility of feeds (Yu, 2005c; Refat, 2018; Carbonaro, 2012).

Little information is available on correlation data between molecular spectral features and metabolic characteristics of faba bean therefore, data related to other feeds including cereals and other legumes are used for matter of comparison in this research. Several studies have shown that rumen degradation kinetics and intestinal digestibility of feeds could be considerably affected by molecular structure changes, demonstrating FTIR could be used as a less expensive, less time consuming, less invasive and simple technique to predict nutrient utilization in dairy cattle and molecular structure changes induced by heat treatments. Multiple regression analyses are a valuable feature to predict nutrient values of different feedstuffs. Both studies are important for further diet formulation, breeding selection programs, prediction of feed quality and establishment of correct processing procedures.

Based on the results from this research, we concluded that even when total protein and carbohydrate contents were similar in low and normal tannin faba bean, difference in protein and CHO fractions were observed, presenting a difference in the degradation behavior of protein and starch in the rumen. The Energy values, total digestible nutrients, and true nutrient supply of low and normal tannin faba bean were similar and consequently the predicted production performance (FMV) was not different. The present study has also provided a more precise understanding of the degradation behavior and intestinal digestion of primary and bypassed nutrients of different genotypes of faba bean with low and normal tannin levels grown in western Canada.

Based on this study, low tannin faba bean can be used as a protein ingredient for dairy cattle due to their degradation characteristics in the rumen and the higher intestinal digestibility of protein than the normal tannin faba bean seeds, therefore they represent a good source of amino acids to be absorbed in the small intestine. Additionally, the low tannin genotypes can be added as a source of energy because of the higher content of rumen undegraded starch and higher amount of total tract digestion of neutral detergent fiber than the normal tannin types.

Normal tannin faba bean seeds also proved to be a good energy and protein source for dairy cattle because of their higher total tract digestion of starch and their higher digestibility in the small intestine compared to the low tannin types; this could provide more available glucose to be absorbed and used for milk production. The condensed tannin content in the normal tannin genotypes showed no effect on rumen degradability of protein in this study.

Heat treatments shifted the site and rate of degradation of feed nutrients, more desirable outcomes were observed with Steam Pressure as this treatment increased the rumen undegradable protein (RUP) and rumen undegradable starch (BST), increasing the digestibility of protein in the small intestine as well. Furthermore, the true nutrient supply (MP) increase caused by Steam Pressure improved the predicted production performance (FMV) of the different faba bean genotypes. Significant correlations were found between protein and CHO spectral profiles and chemical and metabolic characteristics of faba bean which could be used to predict nutritional data in animals. FTIR molecular spectral analysis detected the heat induced molecular structure changes within protein and CHO spectral profiles of low and normal tannin faba bean grown in western Canada. In general, both low and normal tannin types could be a suitable protein and energy source ingredient to combine with other common feedstuffs in diet formulations for cattle.

7. REFERENCES

Acuña, H., Concha, A., and Figueroa, M. 2008. Condensed Tannin Concentrations of Three Lotus Species Grown in Different Environments. Chilean J. Agric. Res. 68: 31-41.

Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D., and Jauncey, K. 2011. Chemical composition and antinutritional factors of field peas (pisum sativum), chickpeas (*Cicer arietinum*), and faba bean (*Vicia faba*) as affected by extrusion preconditioning and drying temperatures. Cereal Chemistry, 88: 80-86. doi: https://doi.org/10.1094/CCHEM-05-10-0077.

Addisu, S. 2016. Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. Online J. Anim. Feed Res. 6: 45-56.

Akibode, S. and Maredia, M. 2011. Global and Regional Trends in Production, Trade and Consumption of Food Legume Crops [Online] Available: https://www.researchgate.net/profile/Mywish_Maredia/publication/266353209_Global_and_Regional_Trends_in_Production_Trade_and_Consumption_of_Food_Legume_Crops/links/5550a13d08ae12808b390271/Global-and-Regional-Trends-in-Production-Trade-and-Consumption-of-Food-Legume-Crops.pdf?origin=publication_detail [2018 Aug 13].

Andrade-Montemayor, H., García, T., and Kawas, J. 2009. Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. R. Bras. Zootec. 38: 277-291.

Association of Official Analytical Chemists, AOAC, 1990. 15th Edition. Official Methods of Analysis. Washington, DC, U.S.A.

Association of Official Analytical Chemists, AOAC, 2005. 18th Edition. Official Methods of Analysis. Gathersburg, MD, U.S.A.

Aykroyd, W. R. and Doughty, J. 1982. Legumes in Human Nutrition. Food & Agriculture Org. Rome, Italy. 152 pp.

Ban, Y. 2016. Molecular Structural, Physiochemical and Nutritional Characterization of New Lines of Brassica Carinata and the Co-products. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, CA.

Berard, N. C., Wang, Y., Wittenberg, K. M., Krause, D. O., Coulman, B. E., McAllister, T. A., and Ominski, K. H. 2011. Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. Can. J. Plant Sci. 91: 669-675. doi:10.4141/CJPS10153.

Bhatty, R. S. 1974. Chemical composition of some faba bean cultivars. Can. J. Plant Sci. 54: 413-421. doi: https://doi.org/10.4141/cjps74-063.

Bianchi, S., Kroslakova, I., and Mayer, I. 2016. Determination of molecular structures of condensed tannins from plant tissues using HPLC-UV combined with thiolysis and MALDI-TOF. [Online] Available: https://bio-protocol.org/e1975 [2018 Oct 19].

Bunglavan, S. J. and Dutta, N. 2013. Use of tannins as organic protectants of proteins in digestion of ruminants. Livestock Sci. 4: 67-77.

Calabrò, S., Cutrignelli, M.I., Gonzalez, O.J., Chiofalo, B., Grossi, M., Tudisco, R., Panetta, C., and Infascelli, F. 2014. Meat quality of buffalo young bulls fed faba bean as protein source. Meat Sci. 96: 591–596. doi: http://dx.doi.org/10.1016/j.meatsci.2013.08.014.

Calsamiglia, S. and Stern, M. D. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73: 1459-1465. doi: https://doi.org/10.2527/1995.7351459x.

Carbonaro, M., Maselli, P., and Nucara, A. 2012. Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic study. A. Amino Acids. 43: 911–921. doi: 10.1007/s00726-011-1151-4.

Carbonaro, M., Maselli, P., Nucara, A. 2015. Structural aspects of legume proteins and nutraceutical properties. Food Res. International. 76: 19–30. doi: http://dx.doi.org/10.1016/j.foodres.2014.11.007.

CFIA, 2018a. Faba Bean. Crop reports. [Online] Available: http://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/fabae.shtml [2018 Jun. 21].

CFIA, 2018b. Genotypes of Crop Kinds Registered in Canada. [Online] Available: http://www.inspection.gc.ca/active/netapp/regvar/regvar_resultse.aspx?lang=e&Reg=&Kind=FABABEAN&SubKind=FABABEAN&Name=&PNTRadio=All&Rep=&Status=&startDate=&endDate=&btn_submit=Submit [2018 Jun. 21].

Chae, B. J., Han, I. K., and Kim, J. H. 1997. Effects of extrusion conditions of corn and soybean meal on the physico-chemical properties, ileal digestibility and growth of weaned pig. Asian Australas. J. Anim. Sci. 10: 170–177. doi: https://doi.org/10.5713/ajas.1997.170.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., and Duc, G. 2010. Nutritional value of faba bean (*Vicia faba L.*) seeds for feed and food. Field Crops Res. 115: 329-339. doi: https://doi.org/10.1016/j.fcr.2009.09.016.

Cubero, J. I. 2011. The Faba Bean: A Historic Perspective. Grain Legumes No 56. [Online] Available: http://www.legumefutures.de/images/Grain_legumes_56_Faba_bean.pdf [2017 Aug. 01].

CVAS, 2018. Lab procedures. [Online] Available: http://www.foragelab.com/Lab-Services/Forage-and-Feed/Lab-Procedures/ [2018, Oct 19].

Damiran, D., Jonker, A., Zhang, X., Yari, M., McKinnon, J. J., McAllister, T., Abeysekara, S., and Yu, P. 2013. Evaluation of the feed value for ruminants of blends of corn and wheat distillers dried grains. J. Agri. Food Chem. 61: 4387-4395.

Douglas, L., Laviolette-Brown, D., Xinyi, M., Shapka, B., and Yu, Z. 2013. Alberta Faba Bean Producers Manual. Crop Science Capstone. 47 pp.

Eastridge, M. L. 2002. Energy in the New Dairy NRC. [Online] Available: https://dairy.osu.edu/sites/dairy/files/imce/PDF/Feed_PDF/Energy%20New%20Dairy%20NRC.pdf [2017 Sep. 11].

Emiola, I. A. and Gous, R. M. 2011. Nutritional evaluation of dehulled faba bean (*Vicia faba cv. Fiord*) in feeds for weaner pigs. S. Afr. J. Anim. Sci. 41: 79-86.

Etemadi, F., Hashemi, M., Mangan, F. and Weis, S. 2015. Fava Beans. Growers Guide in New England [Online] Available: https://ag.umass.edu/sites/ag.umass.edu/files/research-reports/fava_bean_guide_2.pdf [2017 Feb. 26].

Fakhouri, M. O. and Ramaswamy, H. S. 1993. Temperature uniformity of microwave heated foods as influenced by product type and composition. Food Res. Inter. 26: 89-95.

FAO. 2016. Pulses: Nutritious Seeds for a Sustainable Future. [Online] Available: http://www.fao.org/3/a-i5528e.pdf [2017 Feb. 05].

FAO/IAEA. 2000. Quantification of Tannins in Tree Foliage. IAEA. Vienna, Austria. 25 pp.

FAR, Foundation for Arable Research. 2012. Faba bean - A growers' guide. Templeton, NZ. [Online] Available: https://www.far.org.nz/assets/files/uploads/26313_FAR_focus_8_-_faba_beans.pdf [2017 Sep. 11].

Fleury, D. and Barker, B. 2015. Faba Bean Variety Report 2015/16. [Online] Available: http://saskpulse.com/files/general/151026_Faba_bean_variety_report2.pdf [2017 Feb. 05].

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A. N., and Overton, T. R. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112: 29–78. doi: http://dx.doi.org/10.1016/j.anifeedsci.2003.10.006.

Frejnagel, S., Zdunczyk, Z., and Krefft, B. 1997. The chemical composition and nutritive value of low- and high-tannin faba bean genotypes. J. Anim. Feed Sci. 6: 401–412. doi: https://doi.org/10.22358/jafs/69536/1997.

Frutos, P., Hervás, G., Giráldez, F. J., Mantecó, A. R. 2004. Review. Tannins and ruminant nutrition. Spanish J Agric Res. 2: 191-202.

Gallagher, W. 2009. FTIR Analysis of Protein Structure [Online] Available: http://www.chem.uwec.edu/Chem455_S05/Pages/Manuals/FTIR_of_proteins.pdf. [2018 Oct. 19].

Gargallo, S., Calsamiglia, S., and Ferret, A. 2006. Technical note: A modified three-step *in vitro* procedure to determine intestinal digestion of proteins. J. Anim. Sci. 84: 2163–2167. http://doi.org/10.2527/jas.2004-704

Garrido, A., Gomez-Cabrera, A., Guerrero, J. E., and Marquardt, R. R. 1991. Chemical composition and digestibility *in vitro* of *Vicia faba L.* cultivars varying in tannin content. Anim. Feed Sci. Tech. 35: 205-211.

Gatta, D., Russo, C., Giuliotti, L., Mannari, C., Picciarelli, P., Lombardi, L., Giovannini, L., Ceccarelli, N., Mariotti, L. 2013. Influence of partial replacement of soya bean meal by faba bean or peas in heavy pigs diet on meat quality, residual anti-nutritional factors and phytoestrogen content. Arch. Anim. Nutr. 67: 235-247. doi: 10.1080/1745039X.2013.801137.

Gdala, J. and Buraczewska, L. 1997. Chemical composition and carbohydrate content of several genotypes of faba bean and pea seeds. J. Anim. Feed Sci. 6: 123 – 135. doi: https://doi.org/10.22358/jafs/69510/1997.

Goelema, J. O., Spreeuwenberg, M. A. M., Hof, G., van der Poel, A. F. B., and Tamminga, S. 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba bean and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76: 35 -50. doi: https://doi.org/10.1016/S0377-8401(98)00212-0.

Goelema, J., Smits, A., Vaessen, L., and Wemmers, A. 1999. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. Anim. Feed Sci. Technol. 78: 109–126. doi: https://doi.org/10.1016/S0377-8401(98)00266-1.

González, J. and Andrés, S. 2003. Rumen degradability of some feed legume seeds. Anim. Res. Sci. 52: 17-25. doi: https://hal.archives-ouvertes.fr/hal-00889830.

Government of Saskatchewan. 2018. Growing Faba Bean. [Online] Available: https://www.saskatchewan.ca/business/agriculture-natural-resources-and-industry/agribusiness-farmers-and-ranchers/crops-and-irrigation/field-crops/pulse-crop-bean-chickpea-faba-bean-lentils/faba-bean/growing-faba-bean [2018 Jun. 20].

Guevara, V. H. 2017. Evaluation of Pelleted Products Based on Combination of New Co-Products from Bio-Fuel or Bio-Oil Processing, Pea Screenings and Lignosulfonate Chemical Compound for Ruminant Diets. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, CA.

Hagerman, A. E. 2002. Condensed Tannin Methods. [Online] Available: https://www.users.miamioh.edu/hagermae/Condensed%20Tannin%20Methods.pdf [2017 Sep. 10].

Hagerman, A. E., Robbins, C. T., Weerasuriya, Y., Wilson, T., and Mcarthur, C. 1992. Tannin chemistry in relation to digestion. J. Range Manage. 45: 57-62.

Hedley, C. L. 2001. Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics. CABI Publishing. New York, USA. 322 pp.

Heeg, A. 2017. Faba bean as Protein in Livestock Feed. Fact sheet. [Online] Available: http://www.omafra.gov.on.ca/english/livestock/dairy/facts/16-057.htm [2018 Jan. 16].

Heendeniya, R. G., Christensen, D. A., Manz, D. D., McKinnon, J. J., and Yu, P. 2012. Protein fractionation byproduct from canola meal for dairy cattle. J. Dairy Sci. 95: 4488-4500.

Heinrichs, J., Ishler, V., and Maulfair, D. 2018. Feed Efficiency in Lactating Cows and Relationship to Income Over Feed Costs. Pennstate Extension. [Online] Available: https://extension.psu.edu/feed-efficiency-in-lactating-cows-and-relationship-to-income-over-feed-costs [2018 Jun 21].

Higgs, R. J., Chase, L. E., Ross, D. A., and Van Amburgh, M. E. 2015. Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. J. Dairy Sci. 98: 6340-6360. doi: http://doi.org/10.3168/jds.2015-9379.

Hoover, R. and Sosulski, F. W. 1991. Composition, structure, functionality, and chemical modification of legume starches: a review. Can. J. Physiol. Pharmacol. 69: 79-92.

Huang, X., Khan, N. A., Zhang, X., and Yu, P. 2015. Effects of canola meal pellet conditioning temperature and time on ruminal and intestinal digestion, hourly effective degradation ratio, and potential nitrogen to energy synchronization in dairy cows. J. Dairy Sci. 98: 1–10. doi: http://dx.doi.org/10.3168/jds.2014-9295.

Huang, X., Zhang, H., and Yu, P. 2017. Structural changes on a molecular basis of canola meal by conditioning temperature and time during pelleting process in relation to physicochemical (energy and protein) properties relevant to ruminants. Plos One. 12: 1-19. doi:10.1371/journal. pone.0170173.

Iqbal, Z., Sajid, M. S., Abbas, R. Z., and Sindhu, Z. U. D. 2011. Determination of condensed tannin contents from different plants of kherimurat rangeland (Attock, Pakistan). J. Agric. Soc. Sci. 7: 114–116.

Jansman, A. J. M. 1993. Tannins in Faba Beans (*Vicia Faba L.*)-antinutritional properties in monogastric animals, PhD Thesis. Wageningen Agricultural University. The Netherlands.

Joshi, P. K. and Rao, P. P. 2017. Global pulses scenario: status and outlook. Ann. N.Y. Acad. Sci. 1392: 6-17.

Kostuik, J. 2015. Parkland Crop Diversification Foundation 2014 Annual Report. pp 194-201 in J. Kostuik, S. McEachern, and A. Melnychenko, eds. Fababean Cooperative Variety Trials. doi: 10.13140/RG.2.1.2495.8568.

Lanzas, C., Tedeschi, L. O., Seo, S. and Fox, D. G. 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J. Dairy Sci. 90: 507-521.

Lestingi, A., Francesco, T., Arcangelo, V., De Marzo, D., and Facciolongo, A. 2015. The use of faba bean and sweet lupin seeds alone or in combination for growing lambs. 1. effects on growth performance, carcass traits, and blood parameters. Pakistan J. Zool. 47: 989-996.

Levetin, E., and McMahon, K. 2008. Plants and Society. The McGraw−Hill Companies (5th ed.). McGraw-Hill Higher Education. [Online] Available: http://www.life.illinois.edu/ib/102/Levetin/13. Legumes.pdf [2017 Sep. 10].

Li, X., Zhang, Y., Hannoufa, A., and Yu, P. 2015. Transformation with TT8 and HB12 RNAi constructs in model forage (*Medicago sativa*, Alfalfa) affects carbohydrate structure and metabolic characteristics in ruminant livestock systems. J. Agric. Food Chem. 63: 9590-600. doi: 10.1021/acs.jafc.5b03717.

Licitra, G., Hernandez, T. M., and Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57: 347-358. doi: http://doi.org/10.1016/0377-8401(95)00837-3.

Link, W., Hanafy, M., Malenica, N., Jacobsen, H. J., and Jelenic, S. 2008. Faba bean. Pages 71-88 in C. Kole and T. C. Hall, eds. A Compendium of Transgenic Crop Plants. Wiley-Blackwell Publishing, New York, NY.

Lopes, F., Ruh, K., and Combs, D. K. Validation of an Approach to Predict Total-Tract Fiber Digestibility Using a Standardized *In Vitro* Technique for Different Diets Fed to High-Producing Dairy Cows. J. Dairy Sci. 98: 2596–2602. doi: http://dx.doi.org/10.3168/jds.2014-8665.

Maheri-Sis, N., Eghbali-Vaighan, M., Mirza-Aghazadeh, A., Ahmadzadeh, A.R., Aghajanzadeh-Golshani, A., Mirzaei-Aghsaghali, A., and Shaddel-Telli, A. A. 2011. Effects of microwave irradiation on ruminal dry matter degradation of tomato pomace. Res. J. Biol. Sci. 3: 268-272.

Makkar, H. P. S., Becker, K., Abel, H., and Pawelzik, E. 1997. Nutrient contents, rumen protein degradability and antinutritional factors in some colour- and white-flowering cultivars of *Vicia faba bean*. J Sci. Food Agric. 75: 511-520.

Matsushima, J. K. 2006. History of feed processing. pp 1-16 in Proc. of Cattle Grain Proccessing Symposium. [Online] Available: http://beefextension.com/proceedings/cattle_grains06/06-2.pdf [2018 Mar. 22].

McCartney, D. and Fraser, J. 2010. The potential role of annual forage legumes in Canada: A review. Can. J. Plant Sci. 90: 403-420.

McKnight, D. R. and Macleod, G. K. 1977. Value of whole plant faba bean silage as the sole forage for lactating cows. Can. J. Anim. Sci. 57: 601-603. doi: https://doi.org/10.4141/cjas77-077.

Mostafizar, R. and Yu, P. 2017. Molecular basis of structural make-up of feeds in relation to nutrient absorption in ruminants, revealed with advanced molecular spectroscopy: A review on techniques and models. Appl. Spectrosc. Rev. 52: 653-673. doi: 10.1080/05704928.2017.1295385.

Mustafa, A. F. and Seguin, P. 2003. Characteristics and *in situ* degradability of whole crop faba bean, pea, and soybean silages. Can. J. Anim. Sci. 83: 793-799.

Mustafa, A. F., Christensen, D. A., and McKinnon, J. J. 1998. Effects of moist heat treatment on crude protein composition and degradability of field peas. Can. J. Anim. Sci. 78: 453–456.

Nalle, C. L., Ravindran, G. and Ravindran, V. 2010. Influence of dehulling on the apparent metabolizable energy and ileal amino acid digestibility of grain legumes for broilers. J. Sci. Food Agric. 90: 1227–1231

NRC. 1996. Nutrient Requirements of Beef Cattle, 7th Edition. National Research Council, National Academy of Science, Washington, DC, USA.

NRC. 2001. Nutrient Requirement of Dairy Cattle, 7th Edition. National Research Council, National Academy of Science, Washington, DC, USA.

Nuez-Ortín, W. G., and Yu, P. 2010. Estimation of ruminal and intestinal digestion profiles, hourly effective degradation ratio and potential N to energy synchronization of co-products from bioethanol processing. J. Sci. Food Agric. 90: 2058-2067. doi: http://doi.org/10.1002/jsfa.4052

O'Kiely, P., Mc. Gee, L., and Kavanagh, S. 2017. Faba bean for Cattle and Sheep. Teagasc, Animal & Grassland Research and Innovation Centre. [Online] Available: https://www.teagasc.ie/media/website/crops/crops/Beans_for_ruminants.pdf [2017 Nov. 04].

Olson, M. and Bowness, R. 2014. Faba Bean Production [Online] Available: https://albertapulse.com/wp-content/uploads/2017/05/FabaOlsonBownessLacombe-1.pdf [2017 Jun. 11].

Oplinger, E. S., Putnam, D. H., Doll, J. D., and Combs, S. M. 2017. Alternative Field Crops Manual. Fababean. [Online] Available: https://hort.purdue.edu/newcrop/afcm/fababean.html [2017 Sep. 04].

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agric. Sci. 92: 499-503. doi: https://doi.org/10.1017/S0021859600063048.

Ørskov, E. R., Hovell, F. D., and Mould, F. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5: 195-213.

Penner, C. 2018. Lentil and Faba Bean Situation. [Online] Available: http://saskpulse.com/files/report/171221_January_2018_Updated_WEB.pdf [2018 Jun. 21].

PGDC, 2015. Prairie Grain Development Committee. Lines Supported. [Online] Available: http://www.pgdc.ca/pdfs/pulsespecialcrops/Lines%20Supported%20for%20Registration%20by%20PRCPSC%2025Feb2015.pdf [2018 May 16].

Phelps, S. 2015. Faba Bean Agronomy. [Online] Available: http://saskpulse.com/files/general/151222_Phelps_Fababean_webinar.pdf [2016 Jun. 11].

Phelps, S. 2017. Faba Bean Agronomy. [Online] Available: https://saskpulse.com/files/general/170217_Faba_Bean_Agronomy.pdf [2017 Sep. 20].

Porter, L. J., Hrstich, L. N., and Chan, B. J. 1986. The conversion of proanthocyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry. 25: 223-230. doi: https://doi.org/10.1016/S0031-9422(00)94533-3.

Pulse Canada. 2017. Saskatchewan Pulse Crops Seeding and Variety Guide. [Online] Available: http://proof.saskpulse.com/files/general/16SPG8345_Variety_Data_Booklet_v6_LR3.pdf [2017 Feb. 05].

Pulse Canada. 2018. What is a pulse? [Online] Available: http://www.pulsecanada.com/about-pulse-canada/what-is-a-pulse/ [2018 Oct. 20].

Refat, Basim. 2018. Molecular Structure Features and Nutrient Availability and Utilization of Barley Silage Genotypes with Varying Digestible Structural Carbohydrate in Comparison with A new Short- Season Corn Silage in High-Producing Dairy Cattle. Ph.D. Dissertation. University of Saskatchewan, Saskatoon, SK, CA.

Roe, M. B., Sniffen, C. J., and Chase, L. E. 1990. Techniques for measuring protein fractions in feedstuffs. In Proceedings-Cornell Nutrition Conference for Feed Manufacturers, Ithaca, NY, USA, pp 81-88.

Sadeghi, A. A. and Shawrang, P. 2005. Effects of Microwave Irradiation on ruminal degradability and in vitro digestibility of canola meal. Anim. Feed Sci. Technol. 127: 45–54. doi:10.1016/j.anifeedsci.2005.08.016.

Salazar-Villanea, S., Hendriks, W. H., Bruininx, E. M., Gruppen, H., and van der Poel, A. F. B. 2016. Protein structural changes during processing of vegetable feed ingredients used in swine diets: implications for nutritional value. Nutr. Res. Rev. 29: 126–141 doi:10.1017/S0954422416000056.

SAS institute. 2018. Multivariate methods. [Online] Available: https://www.jmp.com/support/help/14/multivariate-methods.shtml# [2018, Jul. 20].

Saskatchewan Pulse Growers. 2018a. Growing Pulses. [Online] Available: http://saskpulse.com/growing-pulses/faba-beans/seeding/ [2017 Feb. 05].

Saskatchewan Pulse Growers. 2018b. Growing Pulses. [Online] Available: http://herle.ca/wp-content/uploads/2016/12/CDC_snowdrop.pdf [2018 Feb. 10].

Saskatchewan Pulse Growers. 2018c. Growing Pulses. [Online] Available: http://saskpulse.com/files/general/Variety_description_CDC_219-16.pdf [2018 Feb. 10].

Schofield, P., Mbugua, D. M., and Pell, A. N. 2001. Analysis of condensed tannins: a review. Anim. Feed Sci. Technol. 91: 21-40.

Schuster-Gajzágó, I. 2004. Nutritional aspects of legumes. Encyclopedia of Food and Agricultural Sciences, Engineering and Technology Resources. [Online] Available: https://www.eolss.net/sample-chapters/C10/E5-02-02.pdf. [2016 Jun. 21].

Shay, P., Trofymow, J. A., and Constabel, C. P. 2017. An improved butanol-HCl assay for quantification of water-soluble, acetone:methanol soluble, and insoluble proanthocyanidins (condensed tannins). Plant Methods. 13: 63. doi: https://doi.org/10.1186/s13007-017-0213-3.

Sinclair, L. A., Garnsworth, P. C., Newbold, J. R., and Buttery, P. J. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 120: 251-263.

Singh, A. K., Bharati, R. C., Manibhushan, N. C., and Pedpati, A. 2013. An assessment of faba bean (Vicia faba L.) current status and future prospect. Afr. J. Agric. Res. 50: 6634-6641. doi: https://doi.org/10.5897/AJAR2013.7335.

Statistical Analysis System (SAS). 2013. Base SAS® 9.4 Procedures Guide: Statistical Procedures, Second Edition. SAS Institute Inc., Cary, NC, USA.

Strydhorst, S. and Olson, M. 2013. Faba Bean Seeding Management in Alberta. Agrifacts. Agdex 142/22-1 [Online] Available:

https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex14482/$file/142_22-1.pdf?OpenElement [2016 Apr. 03].

Stuart, B. 2004. Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons, Ltd. 221 pp.

Sun, B. Khan, N., and Yu, P. 2018b. Molecular spectroscopic features of protein in newly developed chickpea: Relationship with protein chemical profile and metabolism in the rumen and intestine of dairy cows. Spectrochimi. Acta A: Molecular and Biomolecular Spectroscopy. 196: 168 – 177. doi: https://doi.org/10.1016/j.saa.2018.02.008

Sun, B., Khan, N., Sun, M., Prates, L., and Yu, P. 2018a. Curve-linear relationship between altered carbohydrate traits with molecular structure and truly absorbed nutrient supply to dairy cattle in new hulless barley (*Hordeum vulgare L.).* Anim. Feed Sci. Technol. 235: 177–188. doi: https://doi.org/10.1016/j.anifeedsci.2017.11.014.

Tamminga, S., Brandsma, G. G., van Duinkerken, G., van Vuuren, A. M., and Blok, M. C. 2007. Protein evaluation for ruminants: the DVE/OEB 2007-system. CVB Documentation Report, pp 53-58. Wageningen University, Wageningen, The Netherlands.

Tamminga, S., van Straalen, W. M., Subnel, A. P., Meijer, R. G., Steg, A., Wever, C. J., and Block, M. C. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40: 139-155. doi: https://doi.org/10.1016/0301-6226(94)90043-4.

Tamminga, S., Van Vuuren, A. M., Van der Koelen, C. J., Ketelaar, R. S., and van der Togt, P. 1990. Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Netherlands J. Agric. Sci. 38: 513-526.

Tas, B. M., Taweel, H. Z., Smit, H. J., Elgersma, A., Dijkstra, J., and Tamminga, S. 2006. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. J. Dairy Sci. 89: 3494–3500.

Tempel, A. 1982. Tannin-Measuring Techniques: A Review. J. Chem. Ecol. 8: 289–1298. doi: https://doi.org/10.1007/BF00987762.

Theodoridou, K. 2010. The Effects of Condensed Tannins in Sainfoin (*Onobrychis viciifolia*) on its Digestion and Nutritive Value. Ph.D. Thesis. Blaise Pascal University, Clermont-Ferrand, FR.

Theodoridou, K. and Yu, P. 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, and NRC) in nutrient supply and feed milk value (FMV). J. Agric Food Chem. 61: 2820–2830.

Tylutki, T. P., Fox, D. G., Durbal, V. M., Tedeschi, L. O., Russell, J. B., Van Amburgh, M. E., Overton, T. R., Chase, L. E., and Pell, A. N. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Anim. Feed Sci. Technol. 143: 174-202.

Tyrrell, H. F. 2005. Prediction of the Energy Value of Feeds for Lactation. Proc. Southwest Nutr. Conf. [Online] Available: http://www.dairyweb.ca/Resources/USWebDocs/FeedNRG.pdf [2017 Jun. 11].

Van Amburgh, M. E., Chase, L. E., Overton, T. R.; Ross, D. A., Rechtenwald, R. J., Higgs, R. J., and Tylutki, T. P. 2010. Updates to the Cornell Net Carbohydrate and Protein System v6.1 and implications for ration formulation. pp 144-159 in Proceedings of Cornell Nutrition Conference for Feed Manufacturers. Syracuse, NY, USA.

Van Amburgh, M. E., Collao-Saenz, E. A., Higgs, R. J., Ross, D. A., Recktenwald, E. B., Raffrenato, E., Chase, L. E., Overton, T. R., Mills, J. K., and Foskolos, A. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci. 98: 6361-6380. doi: http://doi.org/10.3168/jds.2015-9378.

Van Amburgh, M. E., Foskolos, A., Collao-Saenz, E. A., Higgs, R. J., and Ross, D. A. 2013. Updating the CNCPS feed library with new feed amino acid profiles and efficiencies of use: evaluation of model predictions-version. [Online] Available: https://ecommons.cornell.edu/bitstream/handle/1813/36493/CNC2013_VanAmburgh_m.pdf;sequence=1 [2017 Nov. 13].

Van der Poel, A.F.B., Blonk, J., van Zuilichem, D.J., and van Oort, M.G. 1990. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris*) and effects on protein quality. J. Sci. Food Agric. 53: 215-228.

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597. doi: http://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Wang, X., Liu, G., Ou, Q., Zhao, X., Hao, J., and Zhou, X. 2013. FTIR Study of white and green broad beans based on curve fitting. Asian Agric. Res. 5:1-13.

Wang, Y., Zhang, Y. G., Liu, X., Kopparapu, N. K., Xin, H., Liu, J., and Guo, J. 2015. measurement of the intestinal digestibility of rumen undegraded protein using different methods and correlation analysis. Asian Australas. J. Anim. Sci. 28: 1454-1464. doi: http://dx.doi.org/10.5713/ajas.15.0085.

White, G. A., Smith, L. A., Houdijk, J. C. M., Homer, D., Kyriazakis, I., and Wiseman, J. 2015. Replacement of soy bean meal with peas and faba bean in growing/finishing pig diets: Effect on performance, carcass composition and nutrient excretion. Anim. Feed Sci. Technol. 209: 202-210. doi: https://doi.org/10.1016/j.anifeedsci.2015.08.005.

Xin, H., Falk, K. C., and Yu, P. 2013. Studies on *brassica carinata* seed. 2. carbohydrate molecular structure in relation to carbohydrate chemical profile, energy values, and biodegradation characteristics. J. Agric. Food Chem. 61: 10127–10134. doi: http://dx.doi.org/10.1021/jf402077g.

Xin, H., Qu, Y., Wu, H., Yu, P., and Zhang, Y. 2016. Univariate and multi-variate comparisons of protein and carbohydrate molecular structural conformations and their associations with nutritive factors in typical by-products. J. Sci. Food Agric. 4736–4748. http://dx.doi.org/10.1002/jsfa.7791.

Yan, X., Khan, N. A., Zhang, F., Yang, L., and Yu, P. 2014. Microwave Irradiation induced changes in protein molecular structures of barley grains: relationship to changes in protein chemical profile, protein subfractions, and digestion in dairy cows. J. Agric. Food Chem. 62: 6546-6555.

Yan, X., Zhang, F., and Yu, P. 2017. The inter-relationship between processing-induced molecular structure features and metabolic and digestive characteristics in hulled and hulless barley (Hordeum vulgare) grains with altered carbohydrate traits. J. Sci. Food Agric., 97: 1207-1211. doi:10.1002/jsfa.7851

Yang, L. and Yu, P. 2016. Synchrotron-based and globar-sourced molecular (micro)spectroscopy contributions to advances in new hulless barley (with structure alteration) research on molecular structure, molecular nutrition, and nutrient delivery. Crit. Rev. Food Sci. Nutr. 57: 224-236. doi: http://dx.doi.org/10.1080/10408398.2013.876386.

Yang, L., Christensen, D. A., McKinnon, J. J., Beattie, A. D., and Yu, P. 2013 Effect of altered carbohydrate traits in hulless barley (Hordeum vulgare L.) on nutrient profiles and availability and nitrogen to energy synchronization. J. Cereal Sci. 58: 182-190. doi: http://dx.doi.org/10.1016/j.jcs.2013.05.005.

Ying, Y. 2015. Nutritional and Microstructural Responses in Cereal Grains to Heat-Related Processing Methods. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, CA.

Yu, P. 2005a. Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) microspectroscopy. J. Agric. Food Chem. 53: 7115−7127.

Yu, P. 2005b. Potential protein degradation balance and total metabolizable protein supply to dairy cows from heat-treated faba bean. J. Sci. Food Agric. 85:1268–1274. doi: https://doi.org/10.1002/jsfa.2093.

Yu, P. 2005c. Prediction of protein supply to ruminants from concentrates: comparison of the NRC-2001 model with the DVE/OEB system. J. Sci. Food Agric. 85: 527-538.

Yu, P. 2005d. Protein secondary structures (alpha-helix and beta-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: a new approach. Br. J. Nutr. 94: 655-65.

Yu, P. 2010. Plant-based food and feed protein structure changes induced by gene-transformation, heating and bio-ethanol processing: a synchrotron-based molecular structure and nutrition research program. Mol. Nutr. Food Res. 54: 1535-1545. doi: 10.1002/mnfr.201000178 1535.

Yu, P. 2011. Dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans. J. Dairy Sci. 94: 6092–6102. doi:10.3168/jds.2011-4619.

Yu, P., Christensen, D. A., and McKinnon, J. J. 2003. Comparison of the National Research Council-2001 Model with the Dutch System (DVE/OEB) in the prediction of nutrient supply to dairy cows from forages. J. Dairy Sci. 86: 2178-2192.

Yu, P., Goelema, J. O., and Tamminga, S. 2000. Using the DVE/OEB model to determine optimal conditions of pressure toasting on horse beans (Vicia faba) for the dairy feed industry. Anim. Feed Sci. Technol. 86: 165–176.

Yu, P., Goelema, J. O., Leury, B. J., Tamminga, S. and Egan, A. R. 2002a. An analysis of the nutritive value of heat processed legume seeds for animal production using the DVE/OEB model: a review. Anim. Feed Sci. Technol. 99: 141-176 (Scientific Review Article).

Yu, P., Holmes, J. H. G., Leury, B. J., and Egan, A. R. 1998. Influence of dry roasting on rumen protein degradation characteristics of whole faba bean (*Vicia Faba*) in dairy cows. Asian-Australas J. Anim. Sci. 11: 35-42. doi: https://doi.org/10.5713/ajas.1998.35.

Yu, P., Leury, B. J., and Egan, A. R. 2002b. Ruminal behavior of protein and starch free organic matter of *Lupinus Albus* and *Vicia Faba* in dairy cows. Asian Aust. J. Anim. Sci. 15: 974-981.

Yu, P., McKinnon, J. J., Soita, H. W., Christensen, C. R., and Christensen, D. A. 2005. Use of synchrotron-based FTIR microspectroscopy to determine protein secondary structures of raw and heat- treated brown and golden flaxseeds: A novel approach. Can. J. Anim. Sci. 85: 437–448. doi: https://doi.org/10.4141/A05-004.

Yu, P., Tamminga, S., Egan, A. R., and Christensen, D. A. 2004. Probing equivocal effects of heat processing of legume seeds on performance of ruminants. Aust. J. Anim. Sci. 17: 869-876.

8. APPENDIX

Condensed tannin analysis procedure.

Chemicals and reagents:

Concentrated HCl (36%)

Butanol (n-butanol/1-butanol/butyl alcohol) with CAS71-36-3

HCl Butanol (reagent- 5% HCl + 95% Butanol)

Equipment:

Water bath (Precision, Serial Nº: 602071249, Thermo Scientific, USA)

Spectrophotometer (Spectra max- 384 plus, Molecular devices USA)

Procedure:

1. 20 to 30 mg of ground sample were weighed into a 16 x 125 mm test tubes (in quadruplicates: 4 tubes per sample).
2. 3 ml of HCl butanol reagent were added to all 4 tubes and covered with marbles
3. One test tube (sample blank) of each sample was left at room temperature for 60 min. The rest 3 tubes were incubated in a water bath for 60 min. at 97° C.
4. All tubes were vortexed every 10 min.
5. After 60 min, the tubes were cooled at room temperature with cold water and then centrifuged for 10 min at 3000 rpm.
6. The spectrophometer was turned on 20 min before the reading of the samples and then the wavelength was set at 550 nm. HCl butanol reagent (reagent blank) was used to zero the spectrophotometer.
7. Absorbance (abs) of all solutions including sample blanks were read and results were expressed as absorbance at 550 nm per mg of sample.