INDEPTH STUDY OF THE RELATIONSHIP BETWEEN PROTEIN MOLECULAR STRUCTURE AND THE DIGESTIVE CHARACTERISTICS OF THE PROTEINS IN DRIED DISTILLERS GRAINS WITH SOLUBLES

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ABSTRACT

Dried distillers grains with solubles (DDGS) have been extensively utilized in ruminant rations in western Canada. It is important to ensure the consistent quality of these DDGS. Traditional chemical methods do not consider the inherent structural changes of feed ingredients. The objectives of this study were to investigate the nutritional value of triticale and triticale DDGS in terms of chemical profile, protein and carbohydrate subfractions partitioned using the Cornell Net Carbohydrate and Protein System and energy values calculated according to NRC (2001), to evaluate the digestive characteristics of the proteins in triticale and triticale DDGS using the *in situ* and *in vitro* methods and the DVE/OEB and NRC-2001 models, to identify differences in protein molecular structures between grains (wheat, triticale and corn) and DDGS (wheat DDGS, triticale DDGS, corn DDGS and wheat and corn blend DDGS) using Synchrotron Based Fourier Transform Infrared Microspectroscopy and Diffuse Reflectance Infrared Fourier Transform Spectroscopy and to reveal the relationship between protein molecular structure and protein digestive characteristics in DDGS in dairy cattle. Triticale DDGS was significantly higher ($P<0.01$) in crude protein (31.5 vs. 13.3%), neutral detergent fiber (40.3 vs. 13.5%) and ether extract (6.5 vs. 1.5%) than triticale. There are significant differences in the protein and carbohydrate subfractions ($P<0.05$) and the ruminal degradability of dry matter ($P<0.01$), crude protein ($P<0.01$) and neutral detergent fiber ($P<0.01$) between triticale and triticale DDGS. Triticale and triticale DDGS had similar intestinal digestibility of rumen undegraded crude protein ($P>0.05$). However, triticale DDGS had higher predicted total metabolizable protein ($P<0.01$) and degraded protein balance ($P<0.01$) than triticale. The protein molecular structure study showed significant decreases ($P<0.01$) in the amide I to amide II ratio and the α helix to β sheet ratio from grains to DDGS. Protein digestive characteristics were correlated with protein molecular structures in grains and DDGS and prediction equations were established to estimate protein digestive characteristics of DDGS using protein molecular structure parameters. In conclusion, protein molecular structure varies among different DDGS and their original grains, and this variation is associated with the digestive characteristics of the proteins in the DDGS and their original grains.

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A_I	Area intensity under amide I band (ca. 1718-1579 cm^{-1}) in this study
A_II	Area intensity under amide II band (ca.1579-1488 cm^{-1}) in this study
ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
AECP	Truly absorbed endogenous protein in the small intestine (NRC-2001)
$AMCP^{DVE}$	Truly absorbed microbial protein synthesized in the rumen (DVE/OEB)
$AMCP^{NRC}$	Truly absorbed microbial protein synthesized in the rumen (NRC-2001)
$ARUP^{DVE}$	Truly absorbed rumen undegraded feed protein in the small intestine (DVE/OEB)
$ARUP^{NRC}$	Truly absorbed rumen undegraded feed protein in the small intestine (NRC-2001)
C	Carbon
CA	Rapidly degradable soluble sugar fraction (K_d=200-350% per h) defined by CNCPS
CB1	Intermediately degradable carbohydrate subfraction (K_d=20-50% per h) defined by CNCPS

CB2	Slowly degradable carbohydrate subfraction (K_d=2-10% per h) defined by CNCPS
CC	Unfermentable carbohydrate subfraction defined by CNCPS
CDS	Condensed distillers solubles
EE	Ether extract
CHO	Total carbohydrate
CLA	Cluster analysis
CNCPS	Cornel Net Carbohydrate and Protein System
CP	Crude protein
CRD	Completely randomized design
D	Potential degradable fraction in the *in situ* ruminal incubation
DDG	Dried distillers grains
DDGS	Dried distillers grains with solubles
DE	Digestible energy
DE_{1X}	Digestible energy at maintenance
DE_{3X} (DE_p)	Digestible energy at three times maintenance (i.e. production level)
DM	Dry matter
DOM	Digestible organic matter

DRIFT	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
dRUP	Estimated intestinal digestibility of RUP
DVE	Truly absorbed protein in the small intestine defined by DVE/OEB system
ECP	Endogenous crude protein (NRC-2001)
ED	Effective degradability
EDCP	Effectively degraded crude protein
EDDM	Effectively degraded dry matter
EDNDF	Effectively degraded neutral detergent fiber
ENDP	Endogenous protein loss
FA	Fatty acid
FOM	Fermentable organic matter
FSD	Fourier self deconvolution
FTIR	Fourier Transform Infrared Spectroscopy
h	Hours
H_1630	β sheet peak height (ca. 1630 cm^{-1})
H_1655	α helix peak height (ca. 1655 cm^{-1})
IADP	Estimated intestinally absorbable feed protein
IDP	Estimated intestinal digestibility of RUP

IR	Infrared
K_d	Degradation rate
K_p	Passage rate
MCP	Microbial protein synthesized in the rumen
$MCP_{FOM}{}^{DVE}$	Microbial protein synthesized based on available energy (fermentable organic matter in the DVE/OEB system)
$MCP_{TDN}{}^{NRC}$	Microbial protein synthesized based on available energy (total digestible nutrient in the NRC-2001 model)
MCP_{RDP}	Microbial protein synthesized based on rumen degraded protein
ME	Metabolizable energy
ME_{3X} (ME_p)	Metabolizable energy at three times maintenance (i.e. production level)
MP	Metabolizable protein
N	Nitrogen
NDF	Neutral detergent fiber
NDFn	Nitrogen free neutral detergent fiber (NDFn= NDF – NDICP)
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for growth
NE_{L3X} (NE_{Lp})	Net energy for lactation at three times maintenance (i.e. production level)
NE_m	Net energy for maintenance in growing animals

NFC	Non-fiber carbohydrate
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrate
OM	Organic matter
PA	Non-protein nitrogen defined by CNCPS (K_d is assumed to be infinity)
PB1	Rapidly degradable protein subfraction defined by CNCPS (K_d=120-400% per h)
PB2	Intermediately degradable protein subfraction defined by CNCPS (K_d=3-16% per h)
PB3	Slowly degradable protein subfraction defined by CNCPS (K_d=0.06-0.55% per h
PC	Undegradable protein subfraction defined by CNCPS
PCA	Principal component analysis
DPB^{NRC}	Degraded protein balance (NRC-2001)
OEB	Degraded protein balance (DVE/OEB)
R	Correlation coefficient (based on Pearson or Spearman methods)
R(t)	Feed residue remaining at time t in the *in situ* ruminal incubation
R_I_II	Ratio of amide I to amide II
R_α_β	Ratio of α helix to β sheet

R^2	Coefficient of determination
RCBD	Randomized complete block design
RDP	Rumen degraded protein
RSD	Residual standard deviation
RUDM	Rumen undegraded dry matter
RUNDF	Rumen undegraded neutral detergent fiber
RUP	Rumen undegraded protein
S	Potential soluble fraction in the *in situ* ruminal incubation
SCP	Soluble crude protein
SED	Standard error of difference
SEM	Standard error of mean
SFTIRM	Synchrotron Based Fourier Transform Infrared Microspectroscopy
SSF	Simultaneous saccharification and fermentation
T0	Lag time
TCA	Trichloroacetic acid
tdCP	Total digestible crude protein
tdFA	Total digestible fatty acid
TDN	Total digestible nutrients

TDN_{1X}	Total digestible nutrients at maintenance
TDN_{3X}	Total digestible nutrients at three times maintenance (i.e. production level)
tdNDF	Total digestible neutral detergent fiber
tdNFC	Total digestible non-fiber carbohydrate
TDP	Total digestible feed protein
TP	True protein
U	Potential undegradable fraction in the *in situ* ruminal incubation
UDM	Undigested dry matter

1. General Introduction

The ethanol industry is currently drawing more public attention than at any time in history. The reason for this is that ethanol is a green source of energy with good regeneration ability and competitive pricing (Canadian Renewable Fuels Association, 2011). The energy crisis is becoming a worldwide concern, especially with the increasing prevalence of vehicles which consume a large amount of gasoline and diesel produced from fossil fuels. To alleviate the stressful social and environmental pressure, society has begun to advocate the usage of ethanol as a substitute for fossil fuels (Natural Resources Canada, 2011). Ethanol is mostly produced from feedstocks via fermentation and distillation. The raw materials for the bioethanol industry are mostly sugar and starch crops.

Ethanol production also supplies valuable co-products which have been used as feed ingredients by the feed industry. Dried distillers grains with solubles (DDGS) are the most common of these co-products. The type of DDGS varies with original feedstock used for bioethanol production. The United States and eastern Canada mainly produce corn DDGS, while western Canada produces wheat DDGS. Compared with wheat, triticale, a hybrid grain of wheat and rye, is becoming more economical in western Canada because of lower pricing and similar ethanol yield. Therefore, nutritional information of triticale DDGS is required by the feed industry.

When evaluating the nutritional value of a feed, traditional wet chemical approaches and other chemical based feed evaluation methods are mostly used. Due to technical constraints, the inherent structure of feed ingredients was always neglected by traditional analyses. Recently, infrared spectroscopy techniques have been utilized as a tool to detect the inherent structure of nutrients (e.g. protein, carbohydrate and lipid) in several feedstuffs (e.g. barley, flaxseed and alfalfa) (Doiron et al., 2009a; Liu and Yu, 2010a; Jonker, 2011). However, the molecular structures of the proteins in DDGS are not yet fully understood. Knowledge of the molecular structures may help to improve the quality of DDGS by optimizing bioethanol processing. In addition, this knowledge may contribute to the establishment of more accurate nutritional models.

2. Literature Review

2.1. Bioethanol Industry in Canada

Canadian production of bioethanol reached ca. 1.3 billion liters in 2010, compared with 800 million liters in 2007 (Renewable Fuels Association, 2010). This increase is a result of recent financial investments in the bioethanol industry in Canada. Government policy also supports the development of the bioethanol industry. In 2007, the Government of Canada announced an ecoENERGY program which supported the domestic biofuel industry by investing up to 1.5 billion dollars from 2008 to 2017. The "Ethanol Expansion Program" issued by the Government of Canada also aims to increase the domestic production of ethanol by providing contributions towards the construction of new and existing fuel ethanol production facilities (Natural Resources Canada, 2011). Moreover, the "Renewable Fuels Regulations" published on September 1, 2010 mandates an average 5% of ethanol content in gasoline in Canada effective December 15, 2010 (Natural Resources Canada, 2011).

The substitution of ethanol for gasoline benefits the environment, the economy and society in various ways. Ethanol contains a higher oxygen level (34.7%) compared with gasoline (0%), which makes ethanol a partially oxidized fuel, leading to a lower air to fuel ratio during combustion, meaning less emission and pollution (Otero et al., 2007). In addition, the biomass or grains used for bioethanol production absorb carbon dioxide as they grow. As a result, the net effect is a further reduction in total greenhouse gas emissions (Hill et al., 2006). Approximately 25% more energy is generated than the energy required in bioethanol production and net greenhouse gas emissions are reduced by 12% (Hill et al., 2006). The expansion of the bioethanol industry can also contribute to the rural economy by creating more jobs, stimulating crop production and providing co-products for the feed industry (Government of Alberta, 2008).

There are 16 existing ethanol plants in Canada and most of them utilize feedstocks for ethanol production (Ethanol Producer Magazine, 2011). The substrates utilized by an ethanol plant vary with location. Basically, wheat is the most common substrate for ethanol production in western Canada while the ethanol plants in eastern Canada use corn. Second generation bioethanol production utilizes cellulosic materials, but at present these are not commercially

feasible (Waltz, 2008). Cellulosic ethanol currently accounts for only ca. 2% of total bioethanol production in Canada (Canadian Renewable Fuels Association, 2010).

2.2. Bioethanol Processing Procedures

The principle of bioethanol processing is to convert feedstocks to ethanol via a series of procedures including fermentation, distillation and drying (Nichols and Bothast, 2008). Since most bioethanol facilities use starch-based substrates, the following discussion will only focus on the processing procedures used in starch-based bioethanol production. The conversion from substrate to ethanol is similar for all starch-based feedstocks. Starch is first converted to glucose with the intervention of enzymes. Glucose is fermented into ethanol by yeast (Nichols and Bothast, 2008).

There are two different methods used to convert feedstocks to ethanol including dry grinding and wet milling (Nichols and Bothast, 2008). The dry grinding process grinds the whole kernel of grain for ethanol fermentation in order to get a high ethanol yield (Rausch and Belyea, 2006). The co-products of a dry grinding ethanol plant are carbon dioxide and distillers grains. Wet milling starts with softening corn kernels by soaking the kernels in sulfate dioxide solution for 24 to 48 h and is followed by degermination, grinding and gluten separation. Wet milling can utilize both corn and wheat for ethanol production, although there are differences in the way protein and starch are separated (Graybosch et al., 2009). In US, most wet milling plants utilize corn as their substrate. Corn wet milling produces four major co-products for the feed industry including condensed corn fermented extractives, corn germ meal, corn gluten feed and corn gluten meal (Davis, 2001). In contrast, wheat wet milling exclusively utilizes wheat flour as substrate and produce wheat gluten as a main product (Graybosch et al., 2009). After the protein-starch separation, the processes converting starch to ethanol are the same for corn and wheat wet milling techniques (Graybosch et al., 2009). In Canada, the dry grinding process is currently the predominant method used by ethanol plants.

2.2.1. Grinding and mixing

The first step in the dry grinding process is the grinding of feedstocks either by a hammer mill or a roller mill to crush grain kernels in order to create smaller particles (Rausch and Belyea,

2006; Nichols and Bothast, 2008). The grinding step allows the starch granules to react with enzymes (Nichols and Bothast, 2008). The ground particles will be blended with water forming a slurry which will be cooked. The starch in the slurry will be degraded with the involvement of amylase (Rausch and Belyea, 2006).

2.2.2. Degradation of starch to fermentable sugars

The conversion from starch to ethanol is similar for all grains. Starch consists of two major components namely amylose and amylopectin. In amylose, which is a linear polymer, glucose units are connected by α 1-4 linkages while in amylopectin, which is a larger branched polymer, glucose units are linked by both α 1-4 and α 1-6 linkages (Drapcho et al., 2008). The ratio of amylose to amylopectin in normal starch is 1:3 except for waxy grain varieties where the starch contains about 98% amylopectin (Drapcho et al., 2008).

Prior to fermentation by yeast (i.e. *Saccharomyces cerevisiae*), starch has to be degraded to simple six-carbon sugars via the saccharification process with the participation of heat and enzymes (Power, 2003). Initially, the pH of the slurry should be adjusted to pH 6.0 followed by the addition of the thermostable α-amylase enzyme. Swelling and gelatinization lasts about 30-45 min while the slurry is gradually heated (Drapcho et al., 2008). The slurry is then heated to 110-120°C for 5-7 min using a jet cooker (Bothast and Schlicher, 2005; Drapcho et al., 2008). The starch polymer is broken down into short chain molecules (e.g. dextrins) by the hydrolysis of α 1-4 glucosidic bonds (Nichols and Bothast, 2008). The slurry then leaves the jet cooker and flows into a flash tank in which the temperature falls to 80-90°C. Additional α-amylase is added and the slurry is liquefied for at least 30 min (Bothast and Schlicher, 2005). Other enzymes are sometimes added in conjunction with amylase to achieve a better starch conversion rate, even though these enzymes may not be directly involved in starch degradation. For example, it has been reported that xylanase reduced the viscosity of the mash during ethanol production from sweet potato (Zhang et al., 2010). Some ethanol plants also use cellulase in order to reduce the viscosity in the fermentation of grains other than corn (Ingledew et al., 1999).

2.2.3. Ethanol fermentation

After liquefaction, the temperature of the mixture is decreased to 32°C and the pH is adjusted to about 4.5 (Nichols and Bothast, 2008). Glucoamylase is then added to the slurry to help hydrolyze dextrins into glucose and maltose (Drapcho et al., 2008; Nichols and Bothast, 2008). The slurry is transferred to fermenters where it is referred to as mash. Urea or ammonium sulfate is added as a nitrogen source to promote the growth of yeast.

The addition of the yeast is usually carried out at the same time as glucoamylase is added, resulting in saccharification and fermentation occurring simultaneously in the tank. This fermentation process is termed simultaneous saccharification and fermentation (SSF) (Bothast and Schlicher, 2005). In the SSF process, glucose hydrolyzed from dextrins by glucoamylase can be immediately fermented to ethanol and carbon dioxide by yeast. Carbon dioxide, is one of the two major co-products from ethanol production. It can be compressed and delivered to food and beverage companies (Drapcho et al., 2008). Because of the simultaneous reactions in the SSF process, no accumulation of glucose occurs, thus contamination risk, initial osmotic stress for yeast and cost of energy are relatively low (Bothast and Schlicher, 2005). In practice, the maximum yield of ethanol from glucose is not obtained under normal production procedures. Since the yeast consumes glucose for the production of yeast cell mass, and other co-products such as glycerol are also produced, the utilization efficiency is typically 90-93% (Ingledew, 1999). To prevent the efficiency loss caused by bacteria contamination, some ethanol plants use penicillin (Bayrock et al., 2003) or virginiamycin (Hynes et al., 1997) to control bacteria growth.

2.2.4. Ethanol recovery

Following the 40-60 h fermentation process, the concentration of ethanol is only about 12% (w/v), and therefore distillation and dehydration steps are required in order to obtain a higher purity (Nichols and Bothast, 2008). The mash is first heated and ethanol is distillated to form a mixture consisting of ca. 95% ethanol and 5% water (Drapcho et al., 2008). To acquire a higher purity of ethanol, a molecular sieve is used. A concentration of 99.5% of ethanol can be obtained after dehydration (Swain, 2003).

2.2.5. Stillage processing

The residual mixture left after distillation is called whole stillage and exists in a solid and liquid state. Whole stillage contains the starch-free components of the grain, such as fiber, fat and protein. With further processing, whole stillage can be converted to co-products which are a valuable feed ingredients for livestock. Whole stillage is usually not feasible for animals to consume directly because of its high moisture content, although it also contains a considerable amount of oil, fiber, protein and yeast cells (Drapcho et al., 2008). The solid and liquid fractions in the whole stillage are further separated by centrifugation. The supernatant, which is termed thin stillage, is partially (ca. 30%) recycled to the liquefaction process to reduce the usage of water (Kwiatkowski et al., 2006; Nichols and Bothast, 2008). The remaining thin stillage is condensed from ca. 5 to 35% of solids via evaporation to produce a syrup called condensed distillers solubles (CDS) and is then blended with the solid fraction which is called wet distillers grains to form wet distillers grains with solubles (Ganesan et al., 2006; Rausch and Belyea, 2006; Drapcho et al., 2008). Wet distillers grains or wet distillers grains with solubles can be directly fed to livestock (e.g. feedlot cattle). However, due to limited shelf-life and transportation costs, utilization is relatively limited. To solve this problem, wet distillers grains with solubles are dried to ca. 10-12% moisture to produce dried distillers grains with solubles (DDGS) (McAloon et al., 2000; Drapcho et al., 2008).

2.3. Common Feedstocks Used in Starch-based Bioethanol Production

Traditionally, ethanol is produced from sugar or starch-based feedstocks (Bai et al., 2008). The benefit of sugar feedstocks (e.g. sugar cane, sugar beets and fruit crops) is that they are readily fermentable to yeast without pretreatment (Wilkie et al., 2000). However, the relatively high market value of sugar limits its use. Instead, starch-based feedstocks, such as wheat, corn, barley and rice are more widely used by the bioethanol industry (Olar et al., 2004).

2.3.1. Corn

Corn is one of the most prevalent starch-based substrates used for bioethanol in North America (Olar et al., 2004; Kwiatkowski et al., 2006). The United States is the largest producer of corn in the world. In 2005, more than 90% of ethanol production in the US came from corn

and 16% of the national US corn production was used for ethanol production (Urbanchuk, 2006). By 2010, ca. 35% of the total corn acreage in the US was utilized for ethanol production (Urbanchuk, 2011). In comparison, Canadian bioethanol production consumes about 10% of the total national corn production (Canadian Renewable Fuels Association, 2011). US ethanol production has increased 9 billion gallons from 2000 to 2009 and this led to a tremendous demand for increased corn production. As a result, over the same period, corn acreage increased about 10% (ca. 7.2 million acres) with dramatic changes occurring in the period from 2006 to 2008 (USDA, 2010). Both continuously increasing yields and expanding acreages contribute to the dramatic increase in corn production. Between 2006 and 2008, the shifts in the US farm acreage from soybean to corn accounted for much of the increase in corn production, while reduced soybean acreage had to be compensated for by the growth of other crops (Wallander et al., 2011). From 2000 to 2009, corn for ethanol production increased by ca. 3.7 billion bushels, while total corn production only increased by ca. 3.2 billion bushels (Wallander et al., 2011). The increased use of corn for ethanol production was also reflected in the fact that 40% of the increased corn price was attributed to the increasing global demand for ethanol according to the statistics from 2000 to 2007 (Wallander et al., 2011).

Corn contains ca. 10% protein, 4.5% oil and 10-15% fiber and ash as well as 70% starch and this allows the corn-based bioethanol industry to not only produce the desirable amount of ethanol but also high value co-products (Drapcho et al., 2008). Each kg of corn can produce ca. 0.37 liters of ethanol (Pimentel, 2001) and 0.30 kg corn DDGS (Rosentrater, 2005) in the dry grinding process or ca. 0.03 kg corn oil, 0.05 kg corn gluten meal, and 0.24 kg corn gluten feed in the wet milling process (Bothast and Schlicher, 2005).

2.3.2. Wheat

Wheat has a long history of use as the main raw material for whisky and ethanol production (Agu et al., 2006). Wheat has been utilized as one of the main substrates for bioethanol production in several countries (Canada, China, UK and Europe), depending on availability, location, and price (Batchelor et al., 1994; Atlas, 2008; Balat et al., 2008; Dong et al., 2008). In the US and eastern Canada, corn is the main feedstock for bioethanol production, but in western Canada, wheat is more available. Approximately 50% of the 25 million tons of the

annual Canadian wheat production are produced in Saskatchewan (Textor et al., 1998). Statistics show that 73% of the Canadian bioethanol production comes from corn, while wheat accounted for 17% in 2004 (Olar et al., 2004). As wheat contains a comparable starch content with corn (ca. 65%), it is suitable for starch-based bioethanol production. One of the issues of wheat-based bioethanol production is that wheat normally has a higher protein content (ca. 13 vs. 8.5%) than corn, and since wheat protein is insoluble in water, this may decrease the efficiency of processing because of its higher viscosity (Drapcho et al., 2008). However, the excellent nutritional value of the co-products from wheat bioethanol production is highly recognized (Boila and Ingalls, 1994; Ojowi et al., 1997; Mustafa et al., 2000b; Nyachoti et al., 2005; Beliveau and McKinnon, 2008; McKinnon and Walker, 2008; McKinnon and Walker, 2009; Chibisa et al., 2010). Wheat DDGS usually contains more crude protein (CP) than corn DDGS (ca. 39 vs. 32%) and is a good feed ingredient for livestock especially for dairy and beef cattle (Nuez-Ortín and Yu, 2009). The replacement of barley grain by wheat DDGS at a 50% level in cattle diets is feasible (McKinnon and Walker, 2008).

2.3.3. Triticale

Triticale is a man-made hybrid grain produced by crossing wheat and rye (NRC, 1989a). It was first bred in laboratories during the late 19th century. The main growing areas in Canada are Alberta and Saskatchewan (Canadian Grain Commission, 2009). Triticale, which inherits the robustness of rye and the high nutritional composition of wheat, has high disease and pest resistance and high yield (NRC, 1989a). The yield of triticale is about 8-9 tons per hectare, which is similar to wheat. However, the yields of the top varieties of triticale are higher than those of wheat usually by 20-30%, especially for spring varieties (NRC, 1989a). The potential of triticale as a livestock feed have been recognized. Triticale is higher in lysine relative to wheat (Doxastakis et al., 2002), and contains considerable amounts of energy and amino acids. Triticale also produces high silage yields, greater than those from wheat, barley or oat under some circumstances (NRC, 1989a).

Triticale has also been recognized as a great raw material for bioethanol production. Studies in several countries which include Canada, Germany, Poland, and Latvia (Briggs, 2001; Senn and Pieper, 2008; Jansone et al., 2010; Obuchowski et al., 2010) indicate that triticale is

comparable to wheat as a feedstock for ethanol production with comparable ethanol yields and co-product quality. Compared with wheat and rye, the amylolytic activity of triticale's self-contained amylolytic enzymes (mainly amylase) is higher. This may be beneficial to starch degradation in bioethanol processing (Kučerová, 2007). The factors that may affect the efficiency of triticale as a feedstock for bioethanol production are variety and the yield per unit area (Obuchowski et al., 2010). Overall, triticale is very suitable for bioethanol production especially in western Canada, a main production area of triticale.

2.4. Utilization of DDGS in the Feed Industry

Canadian ethanol plants predominantly use the dry grinding procedure and large amounts of co-products (mainly DDGS) are produced that can be used to meet the needs of livestock (Power, 2003). Compared with wet distillers grains with solubles which must be delivered directly from ethanol plants to adjacent farms (e.g. feedlots), DDGS which has a longer shelf life, can be easily stored and transported. Since DDGS has a high fiber content in addition to a high protein content, it is mostly suitable for feeding ruminant animals.

Modifications to dry grinding corn ethanol processing such as the quick germ process, quick germ quick fiber and enzymatic milling processes have been reported (Singh and Eckhoff, 1996, 1997; Singh et al., 1999, 2005; Wahjudi et al., 2000). These modifications aim to introduce additional co-products, such as germ, pericarp and endosperm fiber using a series of procedures including soaking, coarse grinding, protease incubating and sieving before the starch fermentation process (Singh et al., 2005). Also, due to the extraction of fiber during processing, co-products with high protein and low fiber content are available (Rausch and Belyea, 2006). This expands the utilization of DDGS (with a low fiber content) in monogastric animals (e.g. swine and poultry).

There are several types of DDGS available depending on the base cereal grain used for production. Since corn is the predominant substrate for bioethanol production in North America (US and eastern Canada), studies on the utilization of corn DDGS as feed ingredients in both ruminant and monogastric animals have been extensively conducted (Firkins et al., 1985; Ham et al., 1994; NRC, 1996; Fanning et al., 1999; Klopfenstein et al., 2007). The western Canada prairies produce over 85% of the beef cattle and 45% of the swine produced in Canada

(Anonymous, 2010). Thus, wheat DDGS which is also produced in western Canada has also been evaluated as a feed ingredient in both cattle and swine rations (Boila and Ingalls, 1994; Ojowi et al., 1997; Mustafa et al., 2000b; Nyachoti et al., 2005; Beliveau and McKinnon, 2008; McKinnon and Walker, 2008; McKinnon and Walker, 2009; Chibisa et al., 2010).

2.5. Sources of Variation in the Nutritional Value of DDGS

Compared with previous documented data, the nutrient value of DDGS is improving in recent years with the modification of bioethanol fermentation techniques (NRC, 2001; Spiehs et al., 2002; Rausch and Belyea, 2006). However, the inconsistency in nutritional quality of DDGS remains a major issue that prevents accurate ration formulation (Belyea et al., 2004; Shurson, 2005; Kleinschmit et al., 2006, 2007; Schingoethe et al., 2009). Any modification in bioethanol processing procedures may generate different nutritional values for the co-products produced. For instance, researchers found that increasing the ratio of condensed distillers solubles to dried distillers grains resulted in a decrease of crude protein and an increase in fat concentration (Cao et al., 2009). Belyea et al. (2010) reported that differences in fermentation batches caused greater variation in quality than variation due to ethanol plants or periods.

Fermentation efficiency may vary with starch composition, particularly the ratio of amylose to amylopectin (Sharma et al., 2007), which may result in the inconsistent quality of co-products. Some studies found that the color of wheat DDGS (from light to dark) which may result from a different extent of heating during bioethanol processing was related to the nutritional value (Cozannet et al., 2009). Light color DDGS has a higher nutritional value such as a higher ratio of lysine to crude protein (Cozannet et al., 2009; Cozannet et al., 2010). For corn DDGS, the particle size of ground corn may affect the processing conditions such as the extent of fermentation and recovery of co-products (Kelsall and Lyons, 2003). However, the particle size distribution of DDGS after bioethanol processing is not necessarily related to that of ground corn before bioethanol processing (Rausch et al., 2005). Flowability is another concern that affects the consistency of DDGS quality. Due to the poor flowability, compaction happens during storage and shipping (Bhadra et al., 2009), making DDGS products hard to evenly distribute during mixing and diet formulation (Ileleji et al., 2007). To resolve this problem,

modifications in drying process and temperature control (Ganesan et al., 2009) and changing the physical phase (e.g. pelleting) of DDGS have been investigated (Rosentrater and Kongar, 2009).

2.6. Feed Evaluation

2.6.1. Cornell Net Carbohydrate and Protein System

The Cornell Net Carbohydrate and Protein System (CNCPS) is used to characterize the nutrient value of feed ingredients by dividing crude protein and carbohydrate into several subfractions in order to understand the degradation characteristics during digestion in ruminants (Sniffen et al., 1992; Fox et al., 2004). The predictions by CNCPS include energy and nutrient requirements for maintenance, tissue deposition and milk synthesis, feed intake as well as ruminal degradation of feed carbohydrate and protein fractions, intestinal digestion and excretion. The original division of feed protein and carbohydrate fractions was described in detail by Sniffen et al. (1992). The subfractions are divided based on different degradation rates and calculated based on chemical profiles.

The protein in feed can be partitioned into three fractions including the non-protein nitrogen (PA), true protein (PB) and the unavailable protein (PC). PA is the instantaneously solubilized protein subfraction that is non-protein nitrogen. Fraction PB can be further divided into three subfractions with different ruminal degradation rates. PB1 is the rapidly degraded fraction and is soluble in borate phosphate buffer (K_d = 120-400% per h). PB2 is intermediately degradable protein that consists of neutral detergent soluble protein with intermediate degradation rate (K_d = 3-16% per h) which is believed to partially escape to the small intestine. PB3 consists of protein that is neither soluble in borate phosphate buffer or neutral detergent solution, but is soluble in acid detergent solution. PB3 is associated with the cell wall of plants, thus it is slowly degraded in the rumen (K_d = 0.06-0.55% per h) and most of it escapes to the small intestine. Fraction PC represents protein insoluble in the acid detergent solution and is unavailable to the ruminant (Sniffen et al., 1992).

The carbohydrate in a feed can also be partitioned into four fractions namely CA, CB1, CB2 and CC. CA is a rapidly degradable fraction (K_d = 300% per h), which contains mainly degradable soluble sugars and organic acids, CB1 is an intermediately degradable fraction (K_d =

20-50% per h) consisting of starch and pectin, CB2 is a slowly degradable fraction (K_d = 2-10% per h) which represents the fermentable fiber, while CC is the unavailable cell wall with lignin and resistant starch. Lanzas et al. (2007) modified the carbohydrate subfractions division to eight subfractions by including consideration of volatile fatty acids, lactic acid, other organic acids (e.g. citric, malic and aconitic acids) and soluble fiber.

2.6.2. Energy value estimation in feed ingredients

Estimation of energy content is vital for accurate animal ration formulation. The estimation of energy for the dairy and beef cattle is usually based on a summative calculation of total digestible nutrients (TDN) which can be modeled from the chemical profile of the feed (NRC, 1996, 2001). TDN is calculated from the concentrations of truly digestible non fiber carbohydrate (tdNFC), crude protein (tdCP), fatty acids (tdFA), and neutral detergent fiber (tdNDF) of each feed as follows (Weiss et al., 1992):

tdNFC (%DM) = 0.98 × (100 - [(NDF – NDICP) + CP + EE + Ash]) × PAF,

where 0.98 = expected true digestibility of NFC and PAF = processing adjustment factor that accounts for the effects of processing on starch digestibility.

tdCP (%DM) for concentrates = [1 - (0.4 × (ADICP/CP))] × CP,

tdCP (%DM) for forages = $CP \times e^{[-1.2 \times (ADICP/CP)]}$,

tdFA (%DM) = FA,

where FA (fatty acids) = ether extract (EE) -1.

tdNDF (%DM) = 0.75 × (NDFn - ADL) × [1 - $(ADL/NDFn)^{0.667}$],

where 0.75 = the digestion coefficient for NDF and NDFn = NDF – NDICP.

TDN value is estimated at maintenance (TDN_{1X}) as:

TDN_{1X} = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7,

where 2.25 is the conversion factor from tdFA to digestible carbohydrate and 7 = the estimated metabolic fecal TDN excretion.

For animal products (such as animal protein meals and fat supplements) that contain no cellulose, hemicellulose or lignin, different equations are applied.

In the previous version of NRC (1989b), the combustion heat of TDN_{1X} was directly used to estimate DE, however, considering that different nutrients have different heat combustion values, NRC (2001) calculates apparent DE as:

DE_{1X} (Mcal/kg) = (tdNFC/100 × 4.2) + (tdNDF/100 × 4.2) + (tdCP/100 × 5.6) + (tdFA/100× 9.4) - 0.3,

where 0.3 is obtained by multiplying the estimated metabolic fecal TDN value of 7 by its assumed heat combustion value of 4.4 Mcal/kg DM.

Similar to the TDN_{1X} value, different DE equations for animal protein meals and fat supplements are given by NRC (2001). However, with increases in dry matter intake, the digestibility of diets decreases resulting in a decrease of energy values. Thus, the change caused by different intake levels is adjusted by a discount factor:

Discount = [TDN_{1X} - (0.18 × TDN_{1X} - 10.3)] × Intake / TDN_{1X},

where intake is incremental intake above maintenance and TDN_{1X} is for the entire diet (assumed as 74% for a cow at three time maintenance) not for a single feed ingredient.

Based on the DE_{1X} value and the discount variable, the different energy values of a feed ingredient at different production levels of intake can be calculated according to NRC (1996, 2001):

For dairy cattle,

DE_p (Mcal/kg DM) = DE_{1X} × Discount,

if EE > 3%,

ME_p (Mcal/kg DM) = [(1.01 × DE_p) - 0.45] + 0.0046 × (EE - 3),

if EE < 3%,

ME_p (Mcal/kg DM) = (1.01 × DE_p) - 0.45,

if EE >3%,

NE_{Lp} (Mcal/kg DM) = 0.703 × ME_p - 0.19 + {[(0.097 × ME_p + 0.19)/97] × [EE - 3]},

if EE < 3%,

NE_{Lp} (Mcal/kg DM) = 0.703 × ME_p - 0.19,

For beef cattle,

ME = DE_{1X} × 0.82,

NE_m (Mcal/kg DM) = (1.37 × ME) - (0.138 × ME^2) + (0.0105 × ME^3) - 1.12,

NE_g (Mcal/kg dm) = (1.42 × ME) - (0.174 × ME^2) + (0.0122 × ME^3) - 1.65.

For fat supplements, the above ME_p, NE_{Lp}, NE_m, and NE_g equations are not appropriate due to different conversion efficiencies.

2.6.3. *In situ* incubation to estimate rumen degradation kinetics

The *in situ* nylon bag technique is widely used to measure rumen digestibility of feeds in ruminant animals (Ørskov and McDonald, 1979; Nocek, 1988), because it is simple and capable of handling a large number of samples at one time. The first order kinetic nonlinear model is used extensively to describe rumen degradation kinetics (Ørskov and McDonald, 1979). Robinson et al. (1986) and Dhanoa (1988) modified the model by involving retention time:

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

where R(t) = the residue after t h incubation (%), S = soluble fraction which is determined from the 0 h incubation, U = undegradable fraction (%), T0 = lag time (h), and K_d = degradation rate (% per h). The effective degradability (ED) of nutrients is calculated according to NRC (2001):

$$ED\ (\%) = S + [(100 - S - U) \times K_d)] / (K_p + K_d),$$

where S = soluble fraction (%), K_p = estimated passage rate of digesta from the rumen (% per h) and it is assumed to be 4.5% per h for forages and 6% per h for concentrates (Tamminga et al., 1994).

2.6.4. Prediction models of truly digestible nutrient supply to dairy cattle

Due to the complicated and unique role of the rumen in dairy cattle, the effect of bioethanol processing procedures on nutritional availability, as well as *in situ* and intestinal digestibility of feeds should be studied. In the rumen, the feed protein is partially broken down into non-protein nitrogen for the synthesis of ruminal microbial protein. This leads to a potential loss of true protein in the feed. The synthesis of microbial protein also requires energy derived from the digestion of carbohydrate in the feed. Therefore, a balance between N and energy supply is important in order to optimize microbial protein synthesis (Tamminga et al., 1994; NRC, 2001).

To predict protein utilization in the small intestine, it is important to understand ruminal digestion behavior which is more complicated than in monogatric animals. Various mathematic models such as PDI (INRA, 1978; Verité and Geay, 1987), ARC (1984), NKJ-NJF (1985), AAT-PVB (Madsen, 1985), AP (NRC, 1985), ADPLS and MF (Varhegyi et al., 1998) have been developed to predict protein supply to the small intestine of ruminants.

As new concepts are developed, they should be reflected in modern protein systems. The DVE/OEB (Tamminga et al., 1994) and NRC-2001 (NRC, 2001) systems are two modern protein evaluation systems which are commonly used with dairy cattle. Both the DVE/OEB and NRC-2001 models predict two important factors including the truly digested and absorbed protein in the small intestine (abbreviated as DVE value in the DVE/OEB system and as metabolizable protein (MP) in the NRC-2001 model) as well as the degraded protein balance (abbreviated as OEB value in the DVE/OEB system and as DPB^{NRC} in the NRC-2001 model). The first factor includes the truly absorbable rumen bypass protein, the truly absorbed microbial protein that is synthesized in the rumen and absorbed in the small intestine and the endogenous protein. The second factor represents the N and energy balance that are important for obtaining optimum microbial protein synthesis. However, the calculations that are used in these two systems are different. Generally, the DVE/OEB system calculates energy supply to the rumen microbes based on fermentable organic matter (FOM) while the NRC-2001 model uses total digestible nutrients (TDN). Differences are also reflected in the way they consider endogenous protein i.e. as losses (DVE/OEB system) versus gains (NRC-2001 model). Detailed comparisons between the two models have been made and it was found that in practice when evaluating concentrates and forages, there were high correlations between the values predicted by the two models despite the significant differences in absolute values (Yu et al., 2003a, b).

2.7. Infrared Spectroscopy Techniques in Feed Science

2.7.1. Infrared spectroscopy

2.7.1.1. Basic principles

Infrared spectroscopy has been a useful analytical tool since the 1940s (Stuart, 2004). With its development and modification, it is capable of analyzing samples in almost any state (e.g. liquid, powder and gas). The fundamental principal followed by infrared spectroscopy is the vibrations of atoms. When infrared radiation passes through a sample, some molecules in the sample will absorb part of the radiation at a particular frequency. The absorbed energy appears as a peak in the spectrum and corresponds to the vibration frequency of the molecule (Stuart, 2004).

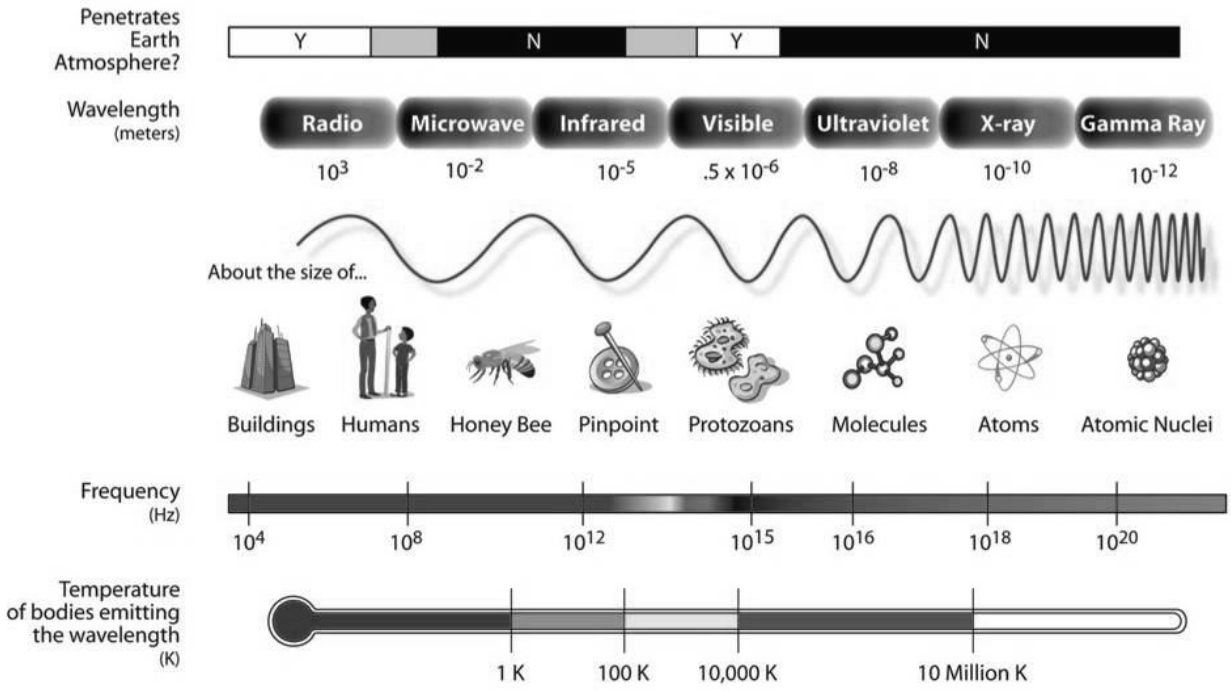


Figure 2.1 The electromagnetic spectrum[1]

[1] Adapt from NASA. 2008. The Electromagnetic Spectrum.

The human eye sees a small part (visible light) of a broad spectrum of electromagnetic radiation (Figure 2.1). Infrared is commonly defined as electromagnetic radiation with frequencies between 14300 and 20 cm^{-1} (McKelvy et al., 1998), and the mid-infrared (Mid-IR) region usually is defined as the region with frequency from ca. 4000-400 cm^{-1} (Messerschmidt and Harthcock, 1988). The absorption of infrared radiation corresponds to energy changes ranging from 8 to 40 kJ/mole (Pavia et al., 2009). The radiation in this energy range also corresponds to the range of vibrational (e.g. stretching and bending) frequencies of covalent bonds in molecules (Pavia et al., 2009).

The vibrations in the bonds of a molecule can be categorized by two models either stretching or bending. Stretching is a vibration model that appears as a change in bond length while bending is one that involves a change in bond angle. For stretching, there is symmetrical stretching (v_s) and asymmetric stretching (v_{as}) while bending includes in-plane bending vibration (δ), which can be divided into scissoring vibration (δ) and rocking vibration (ρ) as well as out-of-plane bending vibration (γ), which can be separated into wagging vibration (ϖ) and twisting vibration (τ) (Messerschmidt and Harthcock, 1988; Jackson and Mantsch, 2000; Stuart, 2004; Pavia et al., 2009). Generally, asymmetric stretching vibrations require higher frequencies than symmetric stretching vibrations and stretching vibrations require higher frequencies than bending vibrations (Pavia et al., 2009).

The infrared absorption frequency can be affected by two factors, the bond strength and the mass of the bonded atoms (Jackson and Mantsch, 2000; Pavia et al., 2009). In general, triple bonds are stronger than double bonds and a single bond between the same two atoms and also have higher frequencies of vibrations, consequently higher wavenumbers (Pavia et al., 2009). The bonds between atoms with lower masses have higher vibrational frequencies (Messerschmidt and Harthcock, 1988; Pavia et al., 2009).

2.7.1.2. Identification of major bands

Since infrared spectroscopy can monitor the radiation uptake by certain molecules in a chemical compound at certain frequencies, it is capable of identifying unknown chemical compounds in a sample by analyzing the characteristics of the spectrum (McKelvy et al., 1998; Stuart, 2004). Although there are complicated interactions among atoms within molecules, the

functional groups are not always found to vary widely and many functional groups exhibit characteristics within a relatively narrow region (Hsu, 1997). The band at which certain chemical structures absorb infrared radiation have been assigned to specific classes of compounds (also called group frequencies) (McKelvy et al., 1998; Stuart, 2004). Normally, the Mid-IR spectrum (ca. 4000-400 cm^{-1}) can be divided into four regions including the X-H stretching region (ca. 4000-2500 cm^{-1}) which generally includes O-H, C-H and N-H stretching, triple bond region (ca. 2500-2000 cm^{-1}), the double-bond region (ca. 2000-1500 cm^{-1}) and the fingerprint region (ca. 1500-600 cm^{-1}) (Stuart, 2004). The detailed assignments of common functional bonds in these bands have been well documented (Stuart, 2004). For example, the C-H bond in the molecule can usually be found within the range ca. 3000 ± 150 cm^{-1} and absorptions in the range of ca. 1715 ± 100 cm^{-1} are normally due to the presence of a C=O bond (Pavia et al., 2009). However, these general descriptions about the specific regions for certain functional compounds are not absolutely accurate as different experimental conditions, sample types and environmental factors could affect the final results (Yu, 2006a; Griffiths and Haseth, 2007)

2.7.1.3. Spectral analysis

The detailed procedures for spectral analysis were comprehensively reviewed by Stuart (2004). The typical infrared absorbance regions for biological compounds (e.g. protein, lipid and carbohydrate) have been well documented (Mantsch and Chapman, 1996; Jackson and Mantsch, 2000; Miller, 2002; Barth and Haris, 2009). For the identification of protein inherent structural characteristics, there are up to nine specific infrared absorption bands which are described as amide A, B and I-VII. However, amide I and II are the most useful bands to reveal changes in the main structures of protein (Krimm and Bandekar, 1986; Surewicz and Mantsch, 1988). The amide I band ranging from ca. 1700 to 1600 cm^{-1} results from 80% C=O stretching (peptide linkage), 10% C–N stretching and 10% N–H bending (Jackson and Mantsch, 1991; Stuart, 2004). The amide I band is found to be highly sensitive to the secondary structural components of protein (Kong and Yu, 2007). The amide II absorbance region can be found from ca. 1600 to 1550 cm^{-1} which consists of 40% C-N stretching associated with 60% N-H in-plane bending (Wetzel, 1993; Stuart, 2004). It is important to note that the amide II band shows less sensitivity to protein secondary structure because it is overlapped with other bands. Therefore, the amide I band can usually be utilized to reveal protein secondary structure with statistical methods, such

as the “2nd derivative” or “Fourier self deconvolution (FSD)”, which could reveal the subcomponent under the amide I band (Stuart, 2004).

2.7.1.3.1. Univariate spectral analysis

Researchers usually use two types of statistical analyses to reveal the biological meaning from the spectra, namely univariate and multivariate methods (Yu, 2005c, 2006b; Liu and Yu, 2010a). The univariate method focuses on quantitative analysis of the mathematical parameters that characterized a spectrum, such as band intensities, integrated intensities, band frequencies and the band intensity ratios (Yu, 2006b). The univariate analysis can help connect the spectra intensity information to the biological meaning on a mathematical basis.

2.7.1.3.2. Multivariate spectral analysis

Compared with univariate statistical analysis which focuses on only one characteristic of one variable at one time, multivariate analysis is capable of evaluating multiple properties of several objectives (Naumann et al., 2009). Hierarchical cluster analysis (CLA) (Jain and Dubes, 1988) and principal component analysis (PCA) (Jolliffe, 1986) can be used to discriminate samples with unknown nature into groups based on similarity in the characteristics of their infrared spectra (Goode et al., 2000; Jackson and Mantsch, 2000; Miller, 2002).

Cluster analysis can distinguish samples with spectral similarity into different groups and display results in dendrograms. The algorithm is performed in a step by step fashion and similarity is defined as the minimal distance between two clusters. First, two spectra with the minimal distance are combined as a cluster. Then, the remaining spectra are resorted and new clusters generated in the next step. With algorithm processing, the distances between all existing clusters are recalculated and resorted stage by stage accordingly and a visible tree diagram (dendrogram) is eventually generated.

Principal component analysis (PCA) is another multivariate analytical tool that can statistically reduce the dataset dimension. Similar to cluster analysis, PCA needs no prior knowledge about the shape of the band (Martin et al., 2004). This method can transform the original data set with a large number of interrelated variables into a new dataset that consists of uncorrelated variables, so called principal components (PCs), while retaining maximal original

variation (Jolliffe, 1986). The first few PCs usually account for most of the original total variances (>95%) and a few PCs are extracted with less and less variances until very little variability or only noise is left (Yu, 2005a). Two-dimensional plots (two PCs) or three-dimensional plots (three PCs) are usually used to graphically display results (Yu, 2005a).

2.7.2. Fourier Transform Infrared Spectroscopy

Before Fourier Transform Infrared Spectroscopy (FTIR) was developed, traditional dispersive infrared spectrometers were used to collect infrared spectra (Stuart, 2004). Compared with dispersive infrared spectrometers, an interferometer (Michelson interferometer) is included in FTIR instruments, which are capable of processing multiple individual scans in a short time. In contrast, dispersive infrared instruments can only record each wavelength across the spectrum one by one and only one scan of the sample is made in a much slower process. In FTIR instruments, the infrared radiation passes through a beam splitter which can split one radiation into two beams to a moving mirror and a fixed mirror, respectively (Anonymous, 2008). When reflections from the two beams are recombined by the beam splitter, they have traveled a different pathlength and an interferogram is generated (Stuart, 2004). The interferogram is a signal that contains distance and frequency information which can be converted by a computer using the Fourier Transform Mathematical Method (Stuart, 2004). The FTIR instrument gives a more representative spectra by combining multiple scans in comparison with one scan from dispersive instruments. Also, greater energy efficiency is achieved during the process because fewer mirrors are used and this leads to a higher signal to noise ratio than dispersive instruments (Anonymous, 2008). With these merits, the FTIR technique has been predominantly used in the infrared spectroscopy field.

2.7.2.1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

Infrared spectroscopy utilizes transmission and reflectance methods to analyze samples depending on the requirements of a study and the phases of samples. For transmission methods, it is suitable to investigate samples in liquid, solid or gaseous forms. For reflectance methods, it is possible to analyze samples that are difficult to investigate by transmission methods (e.g. non-transparent or irregular surfaces) (Stuart, 2004). As one of the reflectance methods, Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) is usually used to detect a

powdered sample, since the radiation passed through particles is reflected in all directions with the combination of reflection, refraction and diffraction (Stuart, 2004; Griffiths and Haseth, 2007).

2.7.2.2. Application of FTIR spectroscopy

Fourier Transform Infrared Spectroscopy has been applied in different academic fields (e.g. chemistry, biology, medical science, food and environmental science). The biological applications of the FTIR technique include the study of a broad range of biomolecules, such as proteins, lipids, nucleic acids, plant or animal tissues and microbes (Jackson and Mantsch, 1995; Mantsch and Chapman, 1996; Stuart, 2004; Barth and Haris, 2009; Naumann et al., 2009; Gordon, 2011). The applications of FTIR on protein structure can be traced back to the 1950s when it was claimed that infrared spectral data can be used to study protein secondary structure (Elliot and Ambrose, 1950). They found close correlations between protein secondary structure and amide bands (Elliot and Ambrose, 1950). With the development of the Fourier Transform Infrared Technique and the modern computer, infrared spectra can be obtained much more rapidly and accurately. X-ray crystallography and nuclear magnetic resonance spectroscopy can provide more detailed information about protein structure such as atom positions. However, they are not suitable for detection of a large number of proteins which is time consuming (Wlodawer et al., 1982; Braun, 1987; Hering and Haris, 2009).

The protein molecular spectra are associated with absorption bands called amide groups (Fabian, 2000; Fabian and Mantele, 2002). There are nine amide bands including amide A, amide B and amides I to VII. However, only amide I and II are frequently used. The best methods to investigate protein secondary structure include Gaussian curve fitting which is used to analyze the multicomponents under the amide I band (Byler and Susi, 1986), Fourier self-deconvolution is also highly effective to identify protein secondary structure and multivariate analyses. Jackson and Mantsch (2000) reviewed the ex vivo tissue related to diagnostical applications of infrared spectroscopy by comparing healthy and diseased tissues on a molecular basis. The FTIR technique can rapidly and accurately reveal the differences between goat and sheep defatted milk without damage to the samples (Pappas et al., 2008).

In feed science, the FTIR technique has been utilized to investigate biopolymer molecular structure in relation to nutrient values, nutrient utilization and availability of feeds. For example, the FTIR spectroscopy has been used to study changes in the structural make-up of lipids and carbohydrates of DDGS (Yu, 2011b; Yu et al., 2011). Different genotypes of barley grains were investigated using FTIR spectroscopy in order to obtain chemical and structural information that might be related to their nutrient degradation behavior in ruminants (Liu and Yu, 2010a). The relationship of the foam stability of different genotypes of alfalfa leaves, to molecular structure of protein and carbohydrate detected by FTIR spectroscopy has also been reported (Jonker, 2011).

2.7.3. Synchrotron Based Fourier Transform Infrared Microspectroscopy

A synchrotron is a cyclic particle accelerator consisting of the an electric field which is used to accelerate particles and a magnetic field which forces the particles to circulate. Synchrotron radiation provides a photon beam ranging from the infrared to x-ray regions. The electromagnetic radiation emitted by a synchrotron facility is extremely bright (Dumas et al., 2007). A synchrotron facility is composed of six main components including an electron gun, linear accelerator, booster ring, storage ring, beamlines and end experimental stations (Canadian Light Source, 2012). The electron gun emits the electrons that are accelerated in the linear accelerator, the booster ring and the circular accelerator. The electrons travel at ca. 99% of the speed of light and are directed into the storage ring. The electrons are then extracted as beamlines which are directly used by the end experiment stations (Dumas and Miller, 2003; Dumas et al., 2007). Synchrotron radiation was first found in an advanced accelerator in April 1947 and was first considered as a useful source of X-rays in the 1950s (Ide-Ektessabi, 2007). After decades of development, the synchrotron has been utilized in various studies such as material science, physics, chemistry and biology (Willmott, 2011).

2.7.3.1. Advantage of the synchrotron

The most important advantage of the synchrotron is that the radiation is an extremely bright light source, much higher than ordinary laboratory bulbs. Compared with conventional thermal infrared sources (e.g. globar) of spectroscopy, the brightness of the synchrotron light is higher by two to three orders of magnitude (Raab and Martin, 2001; Dumas et al., 2007). This

high brightness results from the reduced effective-source size and the narrow range of emission angles (Miller et al., 2000; Miller, 2002). Using synchrotron light as the infrared source of Fourier Transform Infrared Spectroscopy overcomes the limitation of conventional thermal light source with a low degree of diffraction and high signal to noise ratio at high spatial resolutions (Miller et al., 1998; Wetzel et al., 1998; Marinkovic et al., 2002; Miller and Dumas, 2006; Miller et al., 2007). In addition, conventional FTIR fails to investigate the chemical and structural characteristics of a sample in the order of less than 20-50 μm, while synchrotron radiation based FTIR is capable of exploring biological samples in the cellular dimension (5-10 μm) (Miller et al., 1998; Yu, 2006b).

2.7.3.2. Application of SFTIRM in protein structure research

The Synchrotron Based Fourier Transform Infrared Microspectroscopy has extended the application of conventional FTIR spectroscopy by its higher brightness and excellent signal to noise ratio. For example, plant cellular level studies can be conducted, since more accurate imaging and molecular spectra can be obtained (Wetzel et al., 1998; Wetzel et al., 2003; Pietrzak and Miller, 2005). Today, the application of synchrotron science to feed evaluation is increasing with the gradually expanding knowledge of the chemical and structural features of feed. The chemical and structural features of different plant tissues including kernel, root, and vascular bundle sheath have been investigated (Wetzel et al., 1998; Pietrzak and Miller, 2005). However, these studies did not provide information about nutrient values and availability for ingredients for animal feed purpose. Since 2003, a variety of ingredients which are widely involved in animal diets including wheat, barley, corn, canola and flaxseed have been evaluated in terms of chemical and structural information (e.g. chemical functional groups) using SFTIRM (Yu et al., 2003c; Yu et al., 2004c, d; Yu et al., 2005; Yu, 2006b; Yu et al., 2007). Through synchrotron based FTIR microspectroscopy at ultraspatial resolution, the chemical and structural imaging and mapping information (such as the distribution of lignin, cellulose, protein, lipid and carbohydrate in cereal grains) was successfully conducted (Yu et al., 2004b; Yu, 2007; Yu et al., 2007). This information can be related to biodegradation characteristics in animal digestion and is useful for breeders to develop new varieties of plants targeting food or feed uses. Comparisons of different feed-purpose plant (e.g. barley, canola, alfalfa and oat) or animal tissues (e.g. feather) in terms of chemical and structural information and biomolecule functional group have also been made

using this technique (Yu et al., 2004c; Yu et al., 2005; Yu et al., 2008; Yu et al., 2009; Damiran and Yu, 2010; Liu and Yu, 2010b). The SFTIRM has also been applied to detect the relationship between protein molecular structural changes to biodegradation kinetics of flaxseed (treated by different temperatures) in dairy cattle (Doiron et al., 2009a, b). The study reported that heating increased the truly absorbed protein in the small intestine. Recently, a study intending to reveal the molecular spatial distribution and cell wall structure of feed-type sorghum seed was completed (Yu, 2011a).

2.8. Summary

In western Canada, co-products of the bioethanol industry are a very important feed source containing high nutrient values. With the rapid expansion of the bioethanol industry, more and more valuable co-products will be available for feeding livestock in western Canada. The substrates for bioethanol production tend to be diversified depending on the economic requirement. Research has showed that triticale, one of those potentially available substrates, has many merits such as high crop yield, high nutrient values and less competition from human consumption. Triticale has great promise for being one of the more common feedstocks for bioethanol industry use due to these merits. This will provide bioethanol industry producers an alternative choice. Considering the shortcoming of DDGS in maintaining consistent quality during the production process, nutritional information about triticale DDGS is required to improve processing procedures. Thus, the nutrient values of triticale DDGS should be documented in order to meet the need of quality control and accurate ration formulation.

Studies on molecular structural and chemical aspects in feed science offer a new insight which may strongly supplement traditional feed nutrition studies. Since chemical composition does not always explain differences in nutrient utilization and nutrient availability to animals, molecular structures which change under different processing conditions (e.g. heating), could be factors of interest. The Fourier Transform Infrared Spectroscopy and Synchrotron Based Fourier Transform Infrared Spectroscopy could be valuable tools to simply, rapidly and effortlessly obtain biological structure information. Overall, the applications of FTIR and SFTIRM in the feed industry are still at a preliminary stage and more research on different feeds should be done to provide more data for animal nutrition study.

The hypotheses of this study were that bioethanol processing changes protein molecular structures of DDGS in comparison with their original cereal grains; the changes of protein molecular structure between DDGS and original cereal grains affect the protein values and functions in ruminant digestion; protein molecular structure has a relationship to the digestive characteristics of the protein in DDGS and can be used as a predictor to determine the protein nutrient supply.

The objectives of this study were:

- to investigate the nutritional value of triticale DDGS, its chemical profile, carbohydrate and protein subfractions, rumen degradation kinetics, estimated protein intestinal digestion and predicted nutrient supply;
- to identify differences in protein molecular structure from different DDGS and cereal grains using DRFIT and SFTIRM techniques;
- to reveal the relationship between protein molecular structural changes and protein digestive characteristics of DDGS;
- to quantify protein molecular structure in relation to the digestive characteristics of the proteins in dairy cattle;
- to determine whether prediction equations can be built and to identify the most important structural parameters.

3. Comparison of Triticale Grain and Triticale DDGS as Feed Ingredients for Cattle

3.1. Introduction

The ethanol industry in North America is providing a large amount of ethanol and co-products (mainly DDGS) to livestock markets. Due to its availability, wheat has been widely utilized by western Canadian ethanol plants (Wang et al., 1999). The cost of wheat based ethanol production is influenced by wheat price. Recently, wheat prices have risen and the competitive demand from the food industry has increased. As a result, wheat is less competitive as a feedstock for bioethanol production. Grain growers have considered triticale, a hybrid of wheat and rye, as a potential alternative to wheat in western Canada, because of its excellent drought tolerance, high yield and low price (Sosulski et al., 1997; Wang et al., 1997; Chapman et al., 2005). However, current applications of triticale to Canadian agriculture are still not fully developed (Chapman et al., 2005). This situation provides an opportunity for utilization of triticale and raises the interest of both the ethanol and the feed industries as to how triticale will be utilized.

From the aspect of ethanol production, studies on the use of triticale as a substrate for ethanol production have focused on fermentation efficiency, processing techniques and ethanol yield (Wang et al., 1997; Wang et al., 1999). It has been found that triticale has comparable starch content to wheat. As a result, ethanol yield from triticale is similar to that from wheat (Wang et al., 1999). Therefore, the bioethanol industry has been utilizing triticale as an alternative substrate for wheat under circumstances where the price of wheat is unstable.

From the aspect of feed utilization, recent studies have evaluated the effect of triticale DDGS as a feed ingredient for ruminants (Greter et al., 2008; McKeown et al., 2010a, b; Oba et al., 2010; Wierenga et al., 2010) and poultry (Oryschak et al., 2010). These studies confirmed that proper inclusion of different levels of triticale DDGS did not affect livestock performance. However, information with respect to the nutritional value of triticale and triticale DDGS is lacking. This situation is an obstacle to improving the quality of triticale DDGS and to formulating accurate animal diets using triticale DDGS. A database containing nutritional values

for triticale original grain and DDGS would be helpful to reveal the variation in nutritional value, help develop processing methods and consequently produce higher quality triticale DDGS. In addition, the knowledge of ruminal undegradable protein content, intestinal protein digestibility and the degraded protein balance is also crucial to determine the quality of DDGS and should be included in any nutritional evaluation. Expected performance based on modeling animal requirements would be of value for feed evaluation. The Dutch DVE/OEB system (Tamminga et al., 1994) and the NRC-2001 model (NRC, 2001) were developed for such evaluation with dairy cattle.

The hypothesis of this study was that ethanol processing changes the nutrient content and protein digestive characteristics of triticale DDGS relative to triticale grain. The objectives of this study were: 1) to investigate differences in chemical profile, energy values, as well as the protein and carbohydrate fractions between triticale and triticale DDGS; 2) to investigate the ruminal degradation kinetics of various nutrients (DM, CP, NDF) in triticale DDGS and triticale grain; 3) to detect the effect of bioethanol processing procedures on the intestinal availability of the protein in triticale DDGS in comparison with triticale grain; 4) to estimate the amount of truly absorbable protein in the small intestine using the DVE/OEB system and the NRC-2001 model.

3.2. Materials and Methods

3.2.1. Triticale grain and triticale DDGS

Three varieties of spring triticale (Pronghorn, AC Alta and AC Ultima) and three batches of triticale DDGS (Pronghorn, AC Alta and AC Ultima) samples were obtained from Dr. T. McAllister, Alberta Lethbridge Research Centre, Dr. M. Oba, University of Alberta, and Dr. G. McLeod, Agriculture and Agri-food Canada. AC Alta triticale was harvested in 2006, and the Pronghorn and AC Ultima triticales were harvested in 2008. The three batches of triticale DDGS were produced by Alberta Distillers Limited (Calgary, AB) during 2006, 2007 and 2009, respectively.

3.2.2. Animals and diets

Three dry Holstein cows fitted with a rumen cannula with an internal diameter of 10 cm (Bar Diamond, Parma, ID) were used for this work. The cows were cared for according to the guidelines of the Canadian Council on Animal Care (1993). The cows were given *ad libitum* access to water and individually fed 15 kg (as fed) of a totally mixed ration (TMR) twice daily (7.5 kg per feeding) at 0800 and 1600 formulated to meet or exceed NRC Nutrient Requirements (2001). The total mixed ration consisted of 56.82% barley silage, 10.23% alfalfa hay, 4.54% dehydrated alfalfa pellets and 28.41% concentrates (containing barley, wheat, oats, canola meal, soybean meal, wheat DDGS, corn gluten meal, molasses, golden flakes, canola oil, minerals and vitamins) as described in Nuez-Ortín and Yu (2010a).

3.2.3. Rumen incubation procedures

Rumen degradation kinetics were determined using the *in situ* method as described by Yu et al. (2003a). For triticale grain, samples were first coarsely rolled using a Sven Roller Mill (Apolo Machine and Products Ltd., Saskatoon, SK) with a roller gap of 0.203 mm (industry practice). The *in situ* experiment was designed as a Randomized Complete Block Design (RCBD). All treatments were randomly carried out in all three cows in two runs for each incubation time.

Seven gram feed samples were placed into number-marked nylon bags (Nitex 03-41/31 monofilament open mash fabric, Screentec Corp., Mississauga, ON) and tied. The bags were 10 × 20 cm with a pore size of 41µm. The sample bags were placed into a polyester mesh bag (45 × 45 cm with a 90-cm length of rope) and suspended in the rumen. Bags were added into the rumen according to the "gradual addition/all out" schedule and were incubated for 48, 24, 12, 8, 4, 2 and 0 h (Yu et al., 2003a). The number of bags increased with the length of incubation time to ensure sufficient residue for analysis. After incubation, the bags were collected from the rumen, together with the 0 h samples, washed under a cold water stream without detergent to remove the ruminal fluid. Washed bags were dried in a forced air oven at 55°C for 48 h. The dried samples were kept in a refrigerated room (4°C) until needed for chemical analysis.

3.2.4. Chemical analyses

The residues collected from the nylon bags were pooled and transferred into labeled containers and ground through a 1 mm screen (Retsch ZM-1; Brinkmann Instruments, Mississauga, ON) for chemical analysis, with the exception of starch analysis where samples were ground through a 0.5 mm screen. Samples were analyzed for dry matter (DM, AOAC official method 930.15), ash (AOAC official method 942.05), ether extract (EE, AOAC official method 954.02) and crude protein (CP, AOAC official method 984.13) contents according to AOAC (1990). Starch was analyzed using the Megazyme Total Starch Assay Kit (Megazyme International Ltd., Bray, WIC) and by the α-amylase/amyloglucosidase method (McCleary et al., 1997). Acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) were analyzed according to the procedures of Van Soest et al. (1991). Sodium sulfite was added prior to neutral detergent extraction. The N adjusted NDF (NDFn) was calculated as NDF-NDICP. The acid (ADIN) and neutral detergent insoluble N (NDIN) values were determined according to the procedures of Licitra et al. (1996). The non-protein nitrogen (NPN) content was analyzed by precipitating true protein with tungstic acid (samples were soaked in water with 0.3 M Na_2WO_4 for 30 minutes) and calculated as the difference between total N and the N content of the residue after filtration (Licitra et al., 1996). Total soluble crude protein (SCP) was determined by incubating the sample with borate phosphate buffer and filtering through Whatman #54 filter paper (Roe et al., 1990). The non-structural carbohydrates (NSC) including starch, sugars, organic acids, and other reserve carbohydrates such as fructan were estimated by non-fiber carbohydrates and calculated using NRC (2001). The carbohydrate (CHO), true protein, hemicellulose, and cellulose were calculated according to NRC (2001) and Van Soest et al. (1991).

3.2.5. Protein and carbohydrate subfractions

The crude protein and carbohydrate subfractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al., 1992; Chalupa and Sniffen, 1994). For the protein fractions, the total CP pool was partitioned into three categories by this system as fraction PA, PB and PC. Furthermore, PB was sequentially divided into three subfractions named PB1, PB2 and PB3 according to their different degradation rates in the

rumen (Sniffen et al., 1992). Fraction PA is NPN with a hypothesized infinite degradation rate, fraction PB is true protein (TP) which consists of three subfractions with different degradation rates, and fraction PC is the unavailable protein fraction which is ADICP, having an assumed degradation rate of zero because of its high degradation resistance. PB1 is the rapidly degradable fraction of protein with a degradation rate of 120-400% per h, it is soluble in borate phosphate buffer similar to PA and is calculated as SCP minus NPN. PB3 is the plant cell wall associated protein fraction with a degradation rate of 0.06-0.55% per h and is calculated as NDICP minus ADICP. It is insoluble in neutral detergent but soluble in acid detergent solution. It is believed that a large proportion of PB3 can bypass rumen degradation and is available for intestinal digestion. PB2 is calculated as CP minus the sum of PA, PB1, PB3 and PC. It is insoluble in borate phosphate buffer but soluble in neutral detergent. PB2 has a lower degradation rate (3-16% per h) in the rumen than the borate phosphate buffer-soluble fractions (PA and PB1), thus some PB2 fraction escapes from the rumen into the intestine.

Carbohydrate was partitioned into the rapidly degradable fraction (CA) which has a degradation rate of 300% per h, composed of sugars and organic acids, the intermediately degradable fraction (CB1) which is starch and pectin, having an intermediate degradation rate of 20-50% per h, and the slowly degradable fraction (CB2) which is the available cell wall with a degradation rate of 2-10% per h, and an unfermentable fraction (CC) which is the unavailable cell wall. CC is calculated as 0.024 times ADL, CB1 is calculated as NDFn minus CC, CB1 is starch and pectin, and CA is calculated as NFC minus CB1.

3.2.6. Energy values

Estimated energy content was determined using a summative approach (Weiss et al., 1992) from the dairy NRC (NRC, 2001). Total digestible nutrients at maintenance (TDN_{1X}) and digestible energy at maintenance (DE_{1X}) were calculated from total digestible CP (tdCP), fatty acid (tdFA), NDF (tdNDF) and NFC (tdNFC). The change caused by different intake levels was adjusted by a discount factor (NRC, 2001). Based on the DE_{1X} value and the discount variable, digestible energy (DE_{3X}), metabolizable energy (ME_{3X}) and net energy for lactation (NE_{L3X}) at three times maintenance were calculated. Net energy for maintenance (NE_m), and net energy for growth (NE_g) were determined using the beef NRC (NRC, 1996). Both the dairy NRC and beef

NRC use the same formula to estimate NE_m and NE_g. Detailed calculation equations have been reviewed in Chapter 2.

3.2.7. Rumen degradation kinetic model

In situ degradation kinetics for DM, CP and NDF were determined using the first-order kinetics equation described by Ørskov and McDonald (1979) and modified by Robinson et al. (1986) and Dhanoa (1988) to include lag time:

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

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

The degradation model for starch was different in that T0 and U are assumed to be zero in the DVE/OEB system (Tamminga et al., 1994). Therefore,

$$R(t) = (100 - S) \times e^{-K_d \times t},$$

The effective degradability of DM, CP and NDF was calculated according to the following equation:

$$ED\ (\%) = S + D \times K_d/(K_p + K_d),$$

where S = soluble fraction (%); K_p = passage rate (% per h) and was considered to be 6% per h (Tamminga et al., 1994).

The rumen undegradable fraction of DM, CP, NDF and starch were calculated as:

$$RUDM\ (\%) = U + (D \times K_p)/(K_p + K_d),$$

$$RUP\ (\%) = U + (D \times K_p)/(K_p + K_d),$$

$$RUNDF\ (\%) = U + (D \times K_p)/(K_p + K_d),$$

$$RUSt\ (\%) = S \times 0.1 + (D \times K_p)/(K_p + K_d),$$

where D = 100 − S − U (%); K_p is the estimated rate of outflow of digesta from the rumen (% per h) and was assumed to be 6% per h in the DVE/OEB system for concentrate feedstuffs (Tamminga et al., 1994) and the factor 0.1 is a compensation factor between *in situ* and *in vivo* starch results indicating that 10% of the S fraction of starch escapes rumen degradation (Nocek and Tamminga, 1991; Tamminga et al., 1994; Yu et al., 2003a).

3.2.8. *In vitro* estimation of intestinal digestion of rumen undegraded protein

The estimation of intestinal digestion of RUP was determined by a modification of the three-step *in vitro* procedure described by Calsamiglia and Stern (1995). Briefly, dried ground residues containing 15 mg of N after a 12 h ruminal preincubation (Tamminga et al., 1994; Yu et al., 2003a) were exposed for 1 h in 10 mL of 0.1 mol L^{-1} HCl solution containing 1 g L^{-1} of pepsin. The pH was neutralized with 0.5 mL of 1 mol L^{-1} NaOH and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg pancreatin (Sigma-Aldrich, St Louis, MO) was added to the solution and incubated at 38°C for 24 h. After incubation, 3 mL of a 100% (w/v) trichloroacetic acid (TCA) solution was added to stop enzymatic activity and precipitate undigested proteins. Samples were centrifuged and the supernatant (soluble N) was analyzed for N (Kjeldahl method, AOAC 984.13). Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the rumen residue sample.

3.2.9. Predicted metabolizable protein supply in triticale grain and triticale DDGS

3.2.9.1. DVE/OEB system (Non-TDN based model)

The DVE/OEB system was described in detail by Tamminga et al. (1994). The following brief description is provided for the understanding of the concept and prediction of the ruminant nutrient supply. This model features two important values, the DVE value which is the truly absorbed feed protein in the small intestine and the OEB value which stands for the balance between potential microbial protein synthesized based on rumen degraded protein (RDP) and the potential microbial protein synthesized based on energy derived from organic matter fermented in the rumen.

The DVE value was calculated as follows:

DVE (g/kg DM) = $AMCP^{DVE}$ (g/kg DM) + $ARUP^{DVE}$ (g/kg DM) – ENDP (g/kg DM),

where $AMCP^{DVE}$ is the truly absorbable microbial protein synthesized in the rumen, $ARUP^{DVE}$ is the truly absorbed undegraded feed protein in the small intestine, and ENCP is the endogenous protein loss in the small intestine.

The OEB value was calculated as:

OEB (g/kg DM) = $MCP_{RDP}{}^{DVE}$ (g/kg DM) - $MCP_{FOM}{}^{DVE}$ (g/kg DM),

where MCP_{RDP}^{DVE} is microbial protein synthesized from RDP and MCP_{FOM}^{DVE} is microbial synthesized protein from potentially available energy from the fermentation of OM in the rumen. Therefore, a positive OEB indicates a potential N loss from the rumen, while a negative OEB stands for a shortage of N that impairs microbial protein synthesis. The optimal OEB value of a diet is zero or slightly higher than zero (Tamminga et al., 1994).

3.2.9.1.1. Estimation of fermented organic matter

Fermented organic matter (FOM) was used to estimate microbial protein synthesis. According to Tamminga et al. (1994), FOM was calculated as

FOM (g/kg DM) = DOM (g/kg DM) – EE (g/kg DM) – RUP (g/kg DM) – RUSt (g/kg DM) – FP (g/kg DM),

where DOM is digestible OM, estimated after 48 h of incubation, EE = ether extract, RUP = rumen undegraded feed protein, RUSt = rumen undegraded starch, assumed to be zero for the *in situ* residue of DDGS, and FP = end products of fermentation in ensiled forages that are assumed to be zero for concentrates.

3.2.9.1.2. Estimation of microbial protein synthesis in the rumen and truly absorbed rumen microbial protein in the small intestine

Microbial protein synthesis was calculated based on fermented OM as follows:

MCP_{FOM}^{DVE} (g/kg DM) = 0.15 × FOM (g/kg DM),

where 0.15 indicates that 150 g of microbial protein per kg of FOM is assumed to be synthesized (Tamminga et al., 1994).

The DVE/OEB system also considers microbial protein synthesized from RDP (MCP_{RDP}^{DVE}) for the estimation of OEB. The MCP_{RDP}^{DVE} value was calculated as:

MCP_{RDP}^{DVE} (g/kg DM) = CP (g/kg DM) × {1 - [1.11 × RUP (%CP)/100]},

where the factor 1.11 is the regression coefficient between *in situ* RUP and *in vivo* RUP according to the French PDI system (Verité and Geay, 1987).

The truly absorbable microbial protein synthesized in the rumen ($AMCP^{DVE}$) was calculated as:

$AMCP^{DVE}$ (g/kg DM) = 0.75 × 0.85 × MCP_{FOM}^{DVE} (g/kg DM),

where 0.75 means that 75% of the microbial N is present in amino acids while the remainder is present in nucleic acids. The value of 0.85 indicates the true digestibility of microbial protein (Egan et al., 1985).

3.2.9.1.3. Estimation of rumen undegraded feed protein and truly absorbed rumen undegraded feed protein in the small intestine

The content of truly absorbed RUP in the small intestine ($ARUP^{DVE}$) is based on the content and digestibility of ruminally undegraded feed CP (RUP^{DVE}) and was calculated as follows:

$ARUP^{DVE}$ (g/kg DM) = dRUP (%RUP)/100 × RUP^{DVE} (g/kg DM),

RUP^{DVE} (g/kg DM) = 1.11 × RUP (%CP)/100 × CP (g/kg DM),

where dRUP = intestinal digestibility of rumen undegraded protein, 1.11 represents the regression coefficient between *in situ* RUP and *in vivo* RUP according to the French PDI system (Verité and Geay, 1987).

3.2.9.1.4. Estimation of endogenous protein loss in the small intestine

The calculation of DVE requires a correction for endogenous protein loss (ENDP) to account for N lost as a consequence of incomplete digestion. The ENDP is associated with the amount of undigested DM (UDM), which was estimated as:

UDM (g/kg DM) = DM × [(100 – dDM (%)]/100,

where dDM is DM digestibility after a 48 h rumen incubation.

ENDP (g/kg DM) = 0.075 × UDM (g/kg DM),

where 0.075 stands for 75 g of absorbed protein per kg of UDM in feces that is required to compensate for the endogenous protein loss (Tamminga et al., 1994).

3.2.9.2. NRC-2001 model (TDN based model)

The detailed concepts and formulas of the NRC-2001 model are given in NRC (2001). Both the DVE/OEB system and the NRC-2001 model consider the amount of true absorbed protein reaching the small intestine to be an important factor in estimating feed quality. In the NRC-2001 model, this concept is metabolizable protein (MP), which was calculated as:

MP (g/kg DM) = $AMCP^{NRC}$ (g/kg DM) + $ARUP^{NRC}$ (g/kg DM) + AECP (g/kg DM),

where $AMCP^{NRC}$ = absorbable microbial protein synthesized in the rumen, $ARUP^{NRC}$ = truly absorbed bypass feed protein in the small intestine and AECP = truly absorbed endogenous protein in the small intestine. In contrast to the DVE/OEB system, endogenous protein losses are added rather than subtracted from supply.

Although the estimation of rumen degraded protein balance (DPB^{NRC}) is not provided by the NRC-2001 model, it can be calculated based on predicted data and according to the principles of the DVE/OEB system. However, in the NRC-2001 model, DPB^{NRC} is considered as the difference between the potential microbial protein synthesis based on ruminally degraded protein (RDP) and that based on total digestible nutrients (TDN) at a production level rather than on fermentable organic matter (FOM) as in the DVE/OEB system. Therefore:

DPB^{NRC} (g/kg DM) = RDP^{NRC} (g/kg DM) – 1.18 x $MCP_{TDN}{}^{NRC}$ (g/kg DM),

where RDP^{NRC} = rumen degraded protein, and $MCP_{TDN}{}^{NRC}$ = microbial protein synthesis from energy that is provided by total digestible nutrients (discounted at three times maintenance).

3.2.9.2.1. Estimation of total digestible nutrients

The NRC-2001 model requires the TDN_{3X} value in order to estimate rumen microbial protein synthesis. Total digestible nutrient at maintenance (TDN_{1X}) can be calculated according to NRC (2001):

TDN_{1X} = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7,

where 7 represents estimated metabolic fecal TDN. According to NRC (2001), when the intake level increases, TDN declines. Therefore, a discount factor is required to determine TDN_{3X} (NRC, 2001). Assuming the diet TDN at maintenance is 74%, the discount factor at 3 times maintenance (i.e. production level) is 0.918 (NRC, 2001). Therefore, the TDN_{3X} can be calculated as:

$TDN_{3X} = 0.918 \times TDN_{1X}$.

3.2.9.2.2. Estimation of microbial protein synthesis in the rumen and truly absorbed rumen microbial protein in the small intestine

Ruminally synthesized microbial protein is calculated based on discounted TDN and is dependent on the availability of RDP. Thus, MCP^{NRC} was first calculated as follows:

MCP_{TDN}^{NRC} (g/kg DM) = 0.13 × TDN_{3X},

where 0.13 represents 130 g of microbial protein synthesized per kg TDN (discounted) (NRC, 2001).

Then, RDP^{NRC} was calculated as:

RDP^{NRC} (g/kg DM) = CP (g/kg DM) × (100 – RUP (%CP)).

when, RDP^{NRC} > 1.18 × MCP_{TDN}^{NRC}, MCP_{TDN}^{NRC} value is used as MCP^{NRC} for the final $AMCP^{NRC}$ calculation, otherwise, MCP^{NRC} was calculated as:

MCP_{RDP}^{NRC} (g/kg DM) = 0.85 × RDP^{NRC} (g/kg DM),

where 0.85 indicates the amount of RDP converted to microbial protein; and 1.18 results from 1.00/0.85 (NRC, 2001). Since in NRC (2001), both the true protein content of ruminally synthesized microbial CP and the digestibility of ruminally synthesized microbial CP are 0.80, thus, $AMCP^{NRC}$ was estimated as:

$AMCP^{NRC}$ (g/kg DM) = 0.80 × 0.80 × MCP^{NRC} (g/kg DM).

3.2.9.2.3. Estimation of rumen undegraded feed protein and truly absorbed rumen undegraded feed protein in the small intestine

The prediction of $ARUP^{NRC}$ is based on the content and digestibility of RUP^{NRC} and was calculated as:

RUP^{NRC} (g/kg DM) = CP (g/kg DM) × RUP (%CP),

$ARUP^{NRC}$ (g/kg DM) = dRUP (%RUP) × RUP^{NRC} (g/kg DM).

3.2.9.2.4. Estimation of truly absorbed endogenous protein in the small intestine

Endogenous protein losses (ECP) are based on DM content according to NRC (2001). Thus,

ECP (g/kg DM) = 6.25 × 1.9 × DM (%) / 100,

where 6.25 represents the Kjeldahl/N conversion factor; and 1.9 indicates that 1.9 g of endogenous N originated from a kg of DM (NRC, 2001).

It is assumed that 50% of ECP passes to the small intestine of which 80% is true protein (NRC, 2001). Thus, AECP was calculated as:

AECP (g/kg DM) = 0.50 × 0.80 × ECP (g/kg DM).

3.2.10. Statistical analysis

Chemical profile, protein and carbohydrate fractions and estimated energy values. Statistical analyses were performed using the MIXED procedure of SAS (Version 9.1.3). The model used for the analysis was: $Y_{ij} = \mu + F_i + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; F_i was the effect of feed source, as a fixed effect; batch was used as replication and e_{ij} was the random error associated with the observation ij.

***In situ* rumen degradation kinetics, *in vitro* digestion of RUP and predicted nutrient supply.** Statistical analyses were performed using the MIXED procedure of SAS (Version 9.1.3). The model used for the analysis was: $Y_{ijk} = \mu + F_i + S_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ijk; μ was the population mean for the variable; F_i was the effect of feed source as a fixed effect; S_j was the run effect as a random effect; and e_{ijk} was the random error associated with the observation ijk. For all statistical analyses, significance was declared at $P < 0.05$, and trends at $P \leq 0.10$.

3.3. Results and Discussion

3.3.1. Effect of bioethanol processing on chemical profiles of triticale grain and triticale DDGS

Triticale and triticale DDGS chemical profiles are presented in Table 3.1. As expected, chemical profiles were dramatically different between triticale grain and triticale DDGS. Significant differences between triticale and triticale DDGS were found for most nutrients except dry mater content (90.3 vs. 90.3%, P>0.05). Triticale had lower ash (1.7 vs. 4.2%, P<0.01) and ether extract (1.5 vs. 6.5%, P<0.01) than triticale DDGS. For carbohydrate profiles, triticale was lower in NDF (13.5 vs. 40.3%, P<0.01), ADF (3.6 vs. 14.0%, P<0.01) and ADL (5.9 vs. 11.8% NDF, P<0.01), but higher in starch (63.6 vs. 5.2%, P<0.01) than triticale DDGS. The residual starch in DDGS indicated that the fermentation of starch is not complete during bioethanol processing. Triticale had lower values for CP (13.3 vs. 31.5%, P<0.01), NPN (26.2 vs. 100% SCP, P<0.01), NDICP (11.9 vs. 39.9% CP, P<0.01) and ADICP (1.0 vs. 12.0% CP, P<0.01), but a higher value for SCP (33.0 vs. 21.9% CP, P<0.05) than triticale DDGS. The NPN value (100%

SCP) of triticale DDGS is in agreement with that of wheat DDGS as reported by Nuez-Ortín and Yu (2009). The DM (average: 89.7%) for triticale and the DM for triticale DDGS (average: 89.4%) as reported by previous studies (Wang et al., 1997; Wang et al., 1999; McKeown et al., 2010a; Oba et al., 2010) are similar to the results of the current study. However, other analyses were not consistent with previous studies. McKeown et al. (2010a), Oba et al. (2010) and Wierenga et al. (2010) reported lower NDF values (29.6% to 35.6% DM), but the results of the current study are in agreement with Oryschak et al. (2010) who reported a NDF value of 39.4%. Crude protein was similar to the results (average: 31.8%) of previous studies (McKeown et al., 2010a; Oba et al., 2010; Oryschak et al., 2010), but was lower than the 36.7% reported by Wierenga et al. (2010). The ADIN content of triticale DDGS was similar to the value reported by Wierenga et al. (2010) (11.4% of total N) for triticale DDGS and wheat DDGS (average: 10.5% of total N) (Beliveau and McKinnon, 2008). The ether extract value of triticale DDGS was consistent with those reported in all four studies mentioned above. The above comparisons reveal that there is inconsistency in the quality of triticale DDGS derived from different sources.

Table 3.1 Chemical comparison of triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
Basic chemical profile				
Dry matter (%)	90.3	90.3	1.00	0.99
Ash (% DM)	1.7	4.2	0.03	<0.01
Organic matter (% DM)	98.3	95.8	0.03	<0.01
Ether extract (% DM)	1.5	6.5	0.73	<0.01
Carbohydrate and fiber profile				
Starch (% DM)	63.6	5.2	1.21	<0.01
Neutral detergent fiber (% DM)	13.5	40.3	1.56	<0.01
Acid detergent fiber (% DM)	3.6	14.0	0.49	<0.01
Acid detergent lignin (% DM)	0.8	4.7	0.04	<0.01
Acid detergent lignin (% NDF)	5.9	11.8	0.40	<0.01
Crude protein profile				
Crude protein (% DM)	13.3	31.5	1.61	<0.01
Soluble crude protein (SCP, %CP)	33.0	21.9	2.81	<0.05
Non-protein nitrogen (% CP)	8.5	21.9	2.97	<0.05
Non-protein nitrogen (% SCP)	26.2	100.0	3.97	<0.01
Neutral detergent insoluble crude protein (% DM)	1.6	12.6	0.76	<0.01
Neutral detergent insoluble crude protein (% CP)	11.9	39.9	0.39	<0.01
Acid detergent insoluble crude protein (% DM)	0.1	3.7	0.23	<0.01
Acid detergent insoluble crude protein (% CP)	1.0	12.0	1.20	<0.01

SEM = standard error of mean.

Abbreviations: DM, dry matter (%); NDF, neutral detergent fiber (%DM); CP, crude protein (%DM); SCP, soluble crude protein (%CP).

3.3.2. Effect of bioethanol processing on protein and carbohydrate subfractions of triticale grain and triticale DDGS

The carbohydrate and protein in ruminant feeds are generally divided into several subfractions according to the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al., 1992). Protein fractions include PA, PB1, PB2, PB3 and PC. Carbohydrate fractions include CA, CB1, CB2 and CC. Each fraction is believed to have a different degradation rate in the rumen (Sniffen et al., 1992). Significant differences between triticale and triticale DDGS were observed for all protein and carbohydrate subfractions (Table 3.2). Triticale was lower in the rapidly degradable NPN fraction (PA: 8.5 vs. 21.9% CP, $P<0.05$), the slowly degradable CP fraction (PB3: 11.0 vs. 27.9% CP, $P<0.01$) and the unavailable CP fraction (PC: 1.0 vs. 12.0% CP, $P<0.01$) and higher in the rapidly degradable CP fraction (PB1: 24.4 vs. 0% CP, $P<0.01$) and the intermediately degradable CP fraction (PB2: 55.1 vs. 38.3% CP, $P<0.05$) than triticale DDGS. True protein content decreased from 90.5% in triticale to 66.2% in triticale DDGS, indicating overheating during drying. Nuez-Ortín and Yu (2009) compared CNCPS values for wheat, corn, wheat DDGS, and corn DDGS. Their results showed different patterns, a decrease for PA (21.9% vs. 16.3% CP) and no change for true protein (78.1% vs. 78.9% CP) in wheat and wheat DDGS. This inconsistency can be attributed to the different composition of wheat and triticale grain. Wheat has a higher non-protein nitrogen value (PA: 21.9% CP) reported by Nuez-Ortín and Yu (2009) than triticale (PA: 8.5% CP) in this study. Compared with wheat, triticale was significantly lower in non-protein nitrogen and is much higher in rapidly degradable protein (Nuez-Ortín and Yu, 2009).

For the carbohydrate fractions (Table 3.3), triticale is dramatically higher in intermediately degradable carbohydrate (CB1: 76.2 vs. 9.0% CHO, $P<0.01$), but dramatically lower in rapidly degradable sugars (CA: 9.6 vs. 43.0% CHO, $P<0.01$), slowly degradable carbohydrate (CB2: 12.0 vs. 28.4% CHO, $P<0.01$) and unavailable carbohydrate (CC: 2.3 vs. 19.7% CHO, $P<0.01$) than triticale DDGS. The decrease of CB1 value from triticale to triticale DDGS confirms starch was fermented to produce ethanol. However, there is about 5% starch residue left in triticale DDGS which indicates that starch removal during fermentation is not complete. These results are in agreement with a previous study investigating the nutritional variation of wheat, corn, wheat DDGS and corn DDGS (Nuez-Ortín and Yu, 2009). The CC

values of triticale, wheat and corn did not differ. In contrast, both triticale and wheat DDGS have similar CC values which are higher than that of corn DDGS (Nuez-Ortín and Yu, 2009). This finding indicates that corn DDGS is superior to triticale and wheat DDGS in CHO digestion due to the lower undigestible CHO fraction.

Table 3.2 Protein subfractions in triticale grain and dried distillers grains with solubles (DDGS) determined with the Cornell Net Carbohydrate and Protein System (CNCPS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
Protein subfractions (% CP)				
PA	8.5	21.9	2.97	<0.05
PB1	24.4	0.0	1.68	<0.01
PB2	55.1	38.3	3.03	<0.05
PB3	11.0	27.9	1.44	<0.01
PC	1.0	12.0	1.20	<0.01
Protein subfractions (% TP)				
True protein[1] (% CP)	90.5	66.2	1.99	<0.01
PB1 (% TP)	26.9	0.0	1.54	<0.01
PB2 (% TP)	61.0	57.5	3.43	0.52
PB3 (% TP)	12.1	42.5	3.09	<0.01
Protein subfractions (% DM)				
PA	1.1	7.0	1.17	<0.05
PB1	3.3	0.0	0.24	<0.01
PB2	7.3	11.9	0.48	<0.01
PB3	1.5	8.9	0.90	<0.01
PC	0.1	3.7	0.23	<0.01

SEM = standard error of mean.

True protein = PB1 (%CP) + PB2 (%CP) + PB3 (%CP)

Abbreviations: TP, true protein; PA, fraction of CP that is instantaneously solubilized at time zero, calculated as NPN; PB1, rapidly degradable protein fraction that is soluble in borate phosphate buffer and precipitated with trichloroacetic acid, calculated as SCP minus NPN; PB2, intermediately degradable protein fraction calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC; PB3, slowly degradable protein fraction, calculated as NDICP minus ADICP; PC, fraction of undegradable protein, calculated as ADICP. It contained the proteins associated with lignin and tannins and/or heat-damaged proteins such as Maillard reaction products.

Table 3.3 Carbohydrate subfractions in triticale grain and dried distillers grains with solubles (DDGS) determined with the Cornell Net Carbohydrate and Protein System (CNCPS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
Carbohydrate subfractions (% DM)				
CA	8.0	24.8	1.28	<0.01
CB1	63.6	5.2	1.21	<0.01
CB2	10.0	19.4	1.20	<0.05
CC	1.9	11.4	0.10	<0.01
Carbohydrate subfractions (% CHO)				
CHO[1] (% DM)	83.4	57.8	0.96	<0.01
CA (% CHO)	9.6	43.0	1.81	<0.01
CB1 (% CHO)	76.2	9.0	1.50	<0.01
CB2 (% CHO)	12.0	28.4	1.98	<0.01
CC (% CHO)	2.3	19.7	0.40	<0.01

SEM = standard error of mean.

CHO = 100 – crude protein – ether extract – ash

Abbreviations: CHO, carbohydrate; CA, fraction of total carbohydrate with a rapidly K_d (300% per h) and is degradable soluble sugars and organic acids; CB1, fraction of total carbohydrate with an intermediate K_d (20-50% per h); CB2, fraction of total carbohydrate with a slow K_d (2-10% per h) and is available cell wall; CC, fraction of total carbohydrate and is unavailable cell wall and not fermented. CC is calculated as 0.024 times ADL, CB1 is calculated as NDFn minus CC, CB1 is starch and pectin, and CA is calculated as NFC minus CB1.

3.3.3. Effect of bioethanol processing on energy content of triticale grain and triticale DDGS

Triticale was lower in total digestible CP (13.3 vs. 30.0%, P<0.01), total digestible NDF (6.9 vs. 12.0%, P<0.05) and total digestible FA (0.5 vs. 5.5%, P<0.01) while it was higher in total digestible NFC (70.1 vs. 29.5%, P<0.01) than triticale DDGS (Table 3.4). Total digestible nutrients content was higher for triticale than triticale DDGS (84.5 vs. 76.9%, P<0.01). The energy content (estimated by NRC Beef 1996 and NRC Dairy 2001) of triticale and triticale DDGS is presented in Table 3.4. Energy values (DE_{3X}, ME_{3X}, NE_{L3X} for dairy; ME, NE_m and NE_g for beef cattle) were not different (P>0.05) between triticale and triticale DDGS. These results indicate that triticale DDGS has similar energy content to triticale grain, and can replace triticale grain in a dairy or beef diet. It has been reported that corn DDGS has a higher NE_g [1.87 Mcal/kg DM, (Ham et al., 1994); 1.67 Mcal/kg DM, (Nuez-Ortín and Yu, 2009)] than the corn grain (1.48 Mcal/kg DM) (Nuez-Ortín and Yu, 2009). In contrast, energy values of wheat did not change (P>0.05) after fermentation and drying in bioethanol processing (Nuez-Ortín and Yu, 2009).

Table 3.4 Truly digestible nutrients and energy content in triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
Digestible nutrient (% DM)				
tdNFC	70.1	29.5	1.49	<0.01
tdCP	13.3	30.0	1.67	<0.01
tdNDF	6.9	12.0	0.80	<0.05
tdFA	0.5	5.5	0.73	<0.01
Total digestible nutrients (% DM)				
TDN_{1X}	84.5	76.9	1.12	<0.01
Predicted energy values (Mcal/kg DM)				
DE_{3X} (NRC-2001 dairy)	3.42	3.34	0.028	0.10
ME_{3X} (NRC-2001 dairy)	3.01	2.94	0.030	0.20
NE_{L3X} (NRC-2001 dairy)	1.92	1.89	0.024	0.48
ME (NRC-1996 beef)	3.06	2.99	0.025	0.12
NE_m (NRC-1996 beef)	2.08	2.02	0.020	0.11
NE_g (NRC-1996 beef)	1.41	1.36	0.020	0.13

SEM = standard error of mean.

Abbreviations: tdNFC, digestible non-fiber carbohydrate (% DM); tdCP, digestible crude protein (% DM); tdNDF, digestible neutral detergent fiber (% DM); tdFA, digestible fatty acid (% DM); TDN_{1X}, total digestible nutrients at maintenance estimated from NRC dairy model 2001 (% DM); DE_{3X}, digestible energy three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); ME_{3X}, metabolizable energy at three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); NE_{L3X}, Net energy for lactation at three times maintenance estimated from the NRC dairy model 2001 (Mcal/kg DM); ME, metabolizable energy estimated from the NRC beef model 1996 (Mcal/kg DM); NE_m, net energy for maintenance estimated from the NRC beef model 1996 (Mcal/kg DM); NE_g, net energy for growth estimated from the NRC beef model 1996 (Mcal/kg DM).

3.3.4. Effect of bioethanol processing on *in situ* rumen degradability of triticale grain and triticale DDGS

3.3.4.1. Comparison of *in situ* rumen dry matter degradation characteristics between triticale grain and triticale DDGS

For DM degradation characteristics (Table 3.5), triticale was higher ($P<0.05$) in degradation rate (27.1 vs. 10.3% per h), degradable DM fraction (82.7 vs. 50.1%) and EDDM (745 vs. 621 g/kg DM), but lower ($P<0.05$) in S (7.3 vs. 30.5%), U (10.0 vs. 19.4%) and RUDM (255 vs. 379 g/kg DM) than triticale DDGS. According to Herrera-Saldana et al. (1990), K_d for wheat grain was reported as 12.4% per h, which is lower than triticale in this study (27.1% per h). The difference is likely due to the different processing methods utilized for the cereal grain prior to incubation in the rumen. In the current study, the grain was put through a roller mill (gap size 0.203 mm). In the previous study, samples were ground through a 1 mm screen. This resulted in a very high S fraction (61.1%) at 0 h, which is usually eliminated from the K_d calculation. Therefore, K_d was low in that study. In a previous study in which the same *in situ* techniques and same roller milling procedure as in the current study were used, the wheat K_d of DM was 36.7% per h which was higher than the current result (27.13% per h) (Nuez-Ortín and Yu, 2010b). Given that the chemical profile of wheat and triticale are similar, the difference in the rate of DM degradation was likely due to the different inherent structures of different nutrients (e.g. CP, NDF and starch) in the dry matter. Comparing triticale with triticale DDGS, the decreased S value and increased D, K_d and EDDM values demonstrated the same pattern with the previous study on wheat and wheat DDGS (Nuez-Ortín and Yu, 2010b).

Table 3.5 *In situ* rumen degradation kinetics of dry matter in triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
In situ rumen degradation characteristics of DM				
Degradation rate (% per h)	27.1	10.3	1.70	<0.01
Lag time (h)	0.1	0.0	0.04	0.17
Soluble fraction in *in situ* incubation (%)	7.3	30.5	1.25	<0.01
Degradable fraction in *in situ* incubation (%)	82.7	50.1	1.18	<0.01
Undegradable fraction in *in situ* incubation (%)	10.0	19.4	0.74	<0.01
Rumen undegraded feed dry matter (g/kg DM)	255	379	12.4	<0.01
Effectively degraded feed dry matter (g/kg DM)	745	621	12.4	<0.01

SEM = standard error of mean.

3.3.4.2. Comparison of *in situ* rumen crude protein degradation characteristics between triticale grain and triticale DDGS

For CP degradation characteristics (Table 3.6), triticale was higher ($P<0.05$) in K_d (16.9 vs. 9.5% per h) and D (91.1 vs. 64.6%), but lower ($P<0.05$) in S (3.9 vs. 19.3%), U (5.0 vs. 16.1%), RUP (43 vs. 144 g/kg DM) and EDCP (94 vs. 185 g/kg DM) than triticale DDGS. The removal of starch during the bioethanol fermentation led to a 3-fold increase in CP content in the triticale DDGS compared with triticale grain. Therefore, even if the EDCP (%) is decreased from triticale to triticale DDGS, the EDCP (g/kg DM) still increased due to the larger CP content in triticale DDGS. According to the tabular data from NRC (2001), triticale grain has higher K_d (43 vs. 16.9% per h), higher S (51.3 vs. 3.9%), lower D (45.9 vs. 91.1%) and lower U (2.8 vs. 5.0%) for protein than the current results. The difference is likely due to the *in situ* processing method as samples were ground in NRC (2001) vs. coarsely rolled at gap size 0.203 mm in the current study. Compared with the *in situ* data for wheat reported by Herrera-Saldana et al. (1990), both K_d (25.4% per h) and S (72.5% per h) were higher than triticale. Arieli et al. (1995) reported that K_d of wheat was 29.0% per h, which is higher than that of triticale in the current study (16.9% per h). However, EDCP of wheat reported by Arieli et al. (1995) was lower (55.5%) than that of triticale (70.7%) in the current study. The differences were likely generated not only from the different types and genotypes of cereal grains, but also from the processing methods (ground or rolled) used in the experiments. Normally, the smaller the particle size used in the chemical analysis and the *in situ* procedures, the higher the soluble fraction of CP. This is confirmed by comparing the soluble CP (ground through 1 mm screen) content and the *in situ* S fraction (roller gap 0.203 mm) values. The change in the pattern of *in situ* CP degradation kinetics from triticale to triticale DDGS was also observed by Nuez-Ortín and Yu (2010b) who reported similar S and D fractions for wheat and wheat DDGS. The lower proportion of soluble CP in both wheat and wheat DDGS samples in the study of Nuez-Ortín and Yu (2010b) compared with the triticale and triticale DDGS in the present study, along with different bioethanol processing procedures (such as fermentation temperatures and drying period) might contribute to the difference. Compared with triticale DDGS, a similar RUP content (wheat DDGS vs. triticale DDGS: 41.5 vs. 41.4% CP) for wheat DDGS was observed by Ojowi et al. (1997) and Mustafa et al. (2000a), but a higher RUP value (wheat DDGS vs. triticale DDGS: 222 vs. 143 g/kg DM) was reported by Nuez-Ortín and Yu (2010b). It has been concluded that the RUP value was positively correlated to the

ADICP in various sources of DDGS (Boila and Ingalls, 1994; Ham et al., 1994; Mustafa et al., 2000a). The ADICP of triticale DDGS in the current study (12.0% CP) is in agreement with what was observed by Boila and Ingalls (1994), who reported that when ADICP increased from 8.9 to 16.7% CP, RUP also increased.

3.3.4.3. Comparison of *in situ* rumen neutral detergent fiber degradation characteristics between triticale grain and triticale DDGS

For NDF degradation characteristics (Table 3.7), triticale was higher ($P<0.05$) in K_d (14.1 vs. 5.6% per h) and U (48.9 vs. 25.1%), but lower ($P<0.05$) in S (5.2 vs. 19.3%), D (45.9 vs. 56.6%), RUNDF (85 vs. 222 g/kg DM) and EDNDF (50 vs. 181 g/kg DM) than triticale DDGS. Nuez-Ortín and Yu (2010b) reported *in situ* NDF degradation characteristics of wheat grain that were similar in terms of K_d (11.6 vs. 14.1% per h), S (5.9 vs. 5.2%), D (46.4 vs. 45.9%) and EDNDF (50 vs. 50 g/kg DM) to triticale grain. However, wheat DDGS was different from triticale DDGS in terms of higher D (68.5 vs. 56.6%) and lower S (0 vs. 18.3%) and lower EDNDF (107 vs. 181 g/kg DM).

Table 3.6 *In situ* rumen degradation kinetics of crude protein in triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
In situ rumen degradation characteristics of CP				
Degradation rate (% per h)	16.9	9.5	1.14	<0.01
Lag time (h)	0.2	0.0	0.10	0.32
Soluble fraction in *in situ* incubation (%)	3.9	19.3	1.69	<0.01
Degradable fraction in *in situ* incubation (%)	91.1	64.6	1.67	<0.01
Undegradable fraction in *in situ* incubation (%)	5.0	16.1	1.65	<0.01
Rumen undegraded feed protein (% CP)	29.3	41.4	1.79	<0.01
Effectively degraded feed protein (% CP)	70.7	58.6	1.79	<0.01
Rumen undegraded feed protein (g/kg DM) (DVE/OEB)	43	144	4.1	<0.01
Rumen undegraded feed protein (g/kg DM) (NRC 2001)	39	129	3.7	<0.01
Effectively degraded feed protein (g/kg DM)	94	185	9.5	<0.01

SEM = standard error of mean.

Table 3.7 *In situ* rumen degradation kinetics of neutral detergent fiber in triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
In situ rumen degradation characteristics of NDF				
Degradation rate (% per h)	14.1	5.6	0.84	<0.01
Lag time (h)	0.3	0.2	0.21	0.53
Soluble fraction in *in situ* incubation (%)	5.2	18.3	1.82	<0.01
Degradable fraction in *in situ* incubation (%)	45.9	56.6	2.59	<0.05
Undegradable fraction in *in situ* incubation (%)	48.9	25.1	2.54	<0.01
Rumen undegraded feed neutral detergent fiber (% NDF)	62.9	55.2	1.05	<0.01
Effectively degraded feed neutral detergent fiber (% NDF)	37.2	44.8	1.05	<0.01
Rumen undegraded feed neutral detergent fiber (g/kg DM)	85	222	5.4	<0.01
Effectively degraded feed neutral detergent fiber (g/kg DM)	50	181	5.5	<0.01

SEM = standard error of mean.

Table 3.8 Intestinal digestibility and availability of crude protein in triticale grain and dried distillers grains with solubles (DDGS)

	Triticale (n=3)	Triticale DDGS (n=3)	SEM	P values
Protein value				
CP (g/kg DM)	133	315	16.1	<0.01
Rumen phase				
RUP[1] (% CP)	29.3	41.4	1.79	<0.01
RUP^{DVE} (g/kg DM)	43	144	4.1	<0.01
RUP^{NRC} (g/kg DM)	39	129	3.7	<0.01
EDCP (% CP)	70.7	58.6	1.79	<0.01
EDCP (g/kg DM)	94	185	9.5	<0.01
Intestinal phase				
IDP[2] (% RUP)	75.3	72.3	2.72	0.46
IADP[3] (% CP)	21.9	29.8	0.98	<0.01
IADP (g/kg DM)	29	94	3.4	<0.01
TDP[4] (% CP)	92.6	88.4	1.30	<0.05
TDP (g/kg DM)	124	279	11.7	<0.01

SEM = standard error of mean.

[1]Rumen undegraded protein

[2]Estimated intestinal digestibility using the three-step *in vitro* procedure (Calsamiglia and Stern, 1995)

[3]Estimated intestinally absorbable feed protein: IADP = RUP × IDP / 100

[4]Total digestible feed protein: TDP = EDCP + IADP

Abbreviations: CP, crude protein (%DM or g/kg DM); RUP, rumen undegraded feed protein (%CP); RUP, rumen undegraded feed protein (g/kg DM) estimated from the DVE/OEB 1994 model, calculated as 1.11 × CP (g/kg DM) × RUP (%CP); RUP, rumen undegraded feed protein (g/kg DM) estimated from the NRC-2001 model, calculated as CP (g/kg DM) × RUP (%CP); EDCP, effective degradation of feed CP (%CP or g/kg DM); IDP, estimated intestinal digestibility of RUP (%RUP); IADP, estimated intestinally absorbable feed protein (%CP or g/kg DM); TDP, total digestible feed protein (%CP or g/kg DM).

3.3.5. Effect of bioethanol processing on estimated intestinal protein digestion in triticale grain and triticale DDGS

The effects of bioethanol processing on estimated intestinal protein digestibility of triticale and triticale DDGS are shown in Table 3.8. The results show no difference in estimated intestinal digestibility of rumen undegraded protein (IDP) between triticale and triticale DDGS. The estimated intestinally absorbable feed protein (IADP) and the total digestible feed protein (TDP) were higher in triticale DDGS than in triticale grain (IADP: 94 vs. 29 g/kg DM, TDP: 279 vs. 123 g/kg DM). The results indicated that triticale DDGS was a superior source of RUP compared with triticale grain, although the intestinal digestibility of RUP (IDP) in triticale and triticale DDGS were similar. Both IADP and TDP of triticale grain were in good agreement with those of wheat grain (Nuez-Ortín and Yu, 2010b). The IADP of triticale DDGS was lower than that of wheat DDGS (29.8 vs. 44.0% CP), while the TDP values from wheat and triticale DDGS were consistent with each other (Nuez-Ortín and Yu, 2010b). This indicates that wheat and triticale DDGS have different protein digestive characteristics, although their original grains are similar in the ruminal and intestinal availability of protein. This difference is likely due to different processing conditions (e.g. fermentation duration and temperatures) used by different ethanol plants when producing wheat and triticale DDGS.

3.3.6. Prediction of the potential nutrient supply to dairy cattle from triticale grain and triticale DDGS using the DVE/OEB system

The prediction of protein supply from triticale and triticale DDGS to dairy cattle using the DVE/OEB system is presented in Table 3.9. Triticale had a higher FOM value (761 vs. 613 g/kg DM, $P<0.05$), as a result, there was higher $MCP_{FOM}{}^{DVE}$ and higher $AMCP^{DVE}$ values (73 vs. 59 g/kg DM, $P<0.05$) from triticale grain than triticale DDGS. Triticale also had a lower $MCP_{RDP}{}^{DVE}$ value (90 vs. 171 g/kg DM, $P<0.05$) than triticale DDGS. $ARUP^{DVE}$ is lower in triticale than in triticale DDGS (33 vs. 104 g/kg DM, $P<0.05$). This is because no difference was found with regards to dRUP (%RUP, $P>0.05$) between the triticale and triticale DDGS while a lower RUP^{DVE} (43 vs. 144 g/kg DM, $P<0.05$) was found in triticale than triticale DDGS. For endogenous protein losses in the small intestine (ENDP), triticale was lower than triticale DDGS (5 vs. 11 g/kg DM, $P<0.05$). The DVE value for triticale was lower than that for triticale DDGS

(101 vs. 151 g/kg DM, $P<0.05$). While the OEB value was negative for triticale (-24 g/kg DM) and positive for triticale DDGS (79 g/kg DM) ($P<0.05$). Yu et al. (2002) reported FOM values of several feedstuffs were decreased after heating (pressure toasting). However, the decreased FOM value from triticale to triticale DDGS in the current study might mainly result from the removal of starch during bioethanol production. Nuez-Ortín and Yu (2010a) reported a similar trend regarding the DVE and OEB value from wheat to wheat DDGS. Compared with the present study, DVE for wheat DDGS was higher than that for triticale DDGS (249 vs. 151 g/kg DM), but the OEB was similar between them (72 vs. 79 g/kg DM). The higher DVE value of wheat DDGS reported by Nuez-Ortín and Yu (2010a) than that of triticale DDGS in the present study was likely caused by the higher $ARUP^{DVE}$ value (200 vs. 104 g/kg DM) since the $AMCP^{DVE}$ and ENDP were similar.

Table 3.9 Prediction of the potential nutrient supply from triticale grain and dried distillers grains with solubles (DDGS) to dairy cattle determined with the DVE/OEB system

	Feed		SEM	P values
	Triticale (n=3)	Triticale DDGS (n=3)		
Truly absorbed rumen synthesized microbial protein in the small intestine (g/kg DM)				
FOM	761	613	11.6	<0.01
MCP_{FOM}^{DVE} (based on FOM)	114	92	1.8	<0.01
MCP_{RDP}^{DVE}	90	171	9.5	<0.01
$AMCP^{DVE}$	73	59	1.1	<0.01
Truly absorbed rumen undegraded feed protein in the small intestine (g/kg DM)				
RUP^{DVE}	43	144	4.1	<0.01
$ARUP^{DVE}$	33	104	3.8	<0.01
Endogenous protein losses in the digestive tract (g/kg DM)				
DOM	925	822	3.7	<0.01
UDM	64	151	3.7	<0.01
ENDP	5	11	0.3	<0.01
Total truly absorbed protein in the small intestine (g/kg DM)				
DVE (= $AMCP^{DVE}$ + $ARUP^{DVE}$ - ENDP)	101	151	4.2	<0.01
Degraded protein balance (OEB, g/kg DM)				
OEB	-24	79	8.6	<0.01

SEM = standard error of mean.

Abbreviations: FOM, organic matter fermented in the rumen (g/kg DM); MCP_{FOM}^{DVE}, microbial protein synthesized in the rumen based on available energy (g/kg DM); MCP_{RDP}^{DVE}, microbial protein synthesized in the rumen based on available nitrogen (g/kg DM); $AMCP^{DVE}$, truly absorbed microbial protein in the small intestine (g/kg DM); RUP^{DVE}, rumen undegraded feed protein (g/kg DM) estimated from the DVE/OEB 1994 model, calculated as 1.11 × CP (g/kg DM) × RUP (%CP); dRUP, estimated intestinal digestibility of RUP (%RUP); $ARUP^{DVE}$, truly absorbed rumen undegraded protein in the small intestine (g/kg DM); DOM, digestible organic matter (g/kg DM); UDM, undigested dry matter (g/kg DM); ENDP, endogenous protein in the small intestine (g/kg DM); DVE, truly digested protein in the small intestine (g/kg DM); OEB, degraded protein balance (g/kg DM).

3.3.7. Prediction of the potential nutrient supply to dairy cattle from triticale grain and triticale DDGS using the NRC-2001 model

The prediction of protein supply from triticale and triticale DDGS to dairy cattle using the NRC-2001 model is presented in Table 3.10. Triticale had a lower MCP_{RDP}^{NRC} value (80 vs. 158 g/kg DM, $P<0.01$) but a higher MCP_{TDN}^{NRC} value (101 vs. 92 g/kg DM) than triticale DDGS. Since the MCP^{NRC} final value is limited by the lower one of MCP_{RDP}^{NRC} and MCP_{TDN}^{NRC} values, MCP^{NRC} value for the triticale and triticale DDGS is 80 vs. 92 g/kg of DM. The $AMCP^{NRC}$ value increased from triticale (51 g/kg DM) to triticale DDGS (59 g/kg DM). This increase is different from the decreased $AMCP^{DVE}$ values (73 vs. 59 g/kg DM) predicted using the DVE/OEB system. One possible reason is that the DVE/OEB system estimates AMCP exclusively from FOM content, while in the NRC-2001 model, there is a comparison between energy-based MCP and RDP-based MCP. Therefore, the higher MCP_{RDP}^{DVE} value did not account for the increase of $AMCP^{DVE}$. For $ARUP^{NRC}$, there is a significant increase from triticale to triticale DDGS. Considering the similar intestinal digestibility of RUP (dRUP, % RUP), this increase is consistent with $ARUP^{DVE}$ in the DVE/OEB system. Both systems calculate ARUP by multiplying RUP and dRUP (% RUP). Considering the different calculation methods (estimation based on unavailable DM for the DVE/OEB system rather than estimation based on DM of each sample for the NRC-2001 model) for endogenous protein in the two systems, the NRC-2001 model did not distinguish the difference in endogenous protein (AECP) between triticale and triticale DDGS. However, the truly absorbed protein in the small intestine (DVE or MP) and degraded protein balance (OEB or DPB^{NRC}) show consistent results for both systems. The truly absorbed protein in the small intestine is lower in triticale than in triticale DDGS. This indicates that bioethanol processing concentrates the protein and consequently increases the total metabolizable protein. The higher degraded protein balance in triticale DDGS suggests that when formulating diets, other feed ingredients with a lower degraded protein balance should be included in order to achieve optimum protein efficiency.

Table 3.10 Prediction of the potential nutrient supply from triticale grain and dried distillers grains with solubles (DDGS) determined with the NRC-2001 model

	Feed		SEM	P values
	Triticale (n=3)	Triticale DDGS (n=3)		
Truly absorbed rumen synthesized microbial protein in the small intestine (g/kg DM)				
MCP_{RDP}^{NRC} (based on RDP)	80	158	8.1	<0.01
MCP_{TDN}^{NRC} (based on TDN)	101	92	0.9	<0.01
MCP^{NRC}	80	92	1.7	<0.01
$AMCP^{NRC}$	51	59	1.1	<0.01
Truly absorbed rumen undegraded feed protein in the small intestine (g/kg DM)				
RUP^{NRC}	39	129	3.7	<0.01
$ARUP^{NRC}$	29	94	3.4	<0.01
Endogenous protein in the digestive tract (g/kg DM)				
ECP	11	11	0.1	1.00
AECP	4	4	0.0	0.99
Total truly absorbed protein in the small intestine (g/kg DM)				
MP (= $AMCP^{NRC}$ + $ARUP^{NRC}$ + AECP)	85	157	3.5	<0.01
Degraded protein balance (DPB^{NRC}, g/kg DM)				
DPB^{NRC}	-25	77	10.4	<0.01

SEM = standard error of mean.

Abbreviations: MCP_{RDP}^{NRC}, microbial protein synthesized in the rumen based on RDP (g/kg DM); MCP_{TDN}^{NRC}, microbial protein synthesized in the rumen based on discounted TDN (g/kg DM); MCP^{NRC}, microbial protein synthesized in the small intestine (g/kg DM); $AMCP^{NRC}$, truly absorbed microbial protein in the small intestine (g/kg DM); RUP^{NRC}, rumen undegraded feed protein (g/kg DM) estimated from the NRC dairy 2001 model, calculated as CP (g/kg DM) × RUP (%CP); $ARUP^{NRC}$, truly absorbed rumen undegraded protein in the small intestine (g/kg DM); ECP, endogenous protein (g/kg DM); AECP, truly absorbed endogenous protein in the small intestine (g/kg DM); MP, metabolizable protein (g/kg DM); DPB^{NRC}, degraded protein balance (g/kg DM).

3.4. Conclusion

In summary, triticale and triticale DDGS are significantly different in chemical composition and protein and carbohydrate fractions, but similar in energy content estimated by both NRC dairy and beef models. The results indicate bioethanol processing increases the concentration of nutrients in triticale DDGS due to the removal of most of the starch from the whole grain. Similar energy contents suggest triticale DDGS can be a good alternative to triticale grain in ruminant diets. The differences in the rumen and intestinal digestion features and predicted protein supply are attributed to the chemical changes from the original feedstock to DDGS as a result of bioethanol processing. Triticale DDGS provides a higher truly absorbed protein in the small intestine and degraded protein balance for ruminants than the original triticale grain, which indicated that triticale DDGS is a superior source of metabolizable protein than its original grain.

The results in this chapter did not consider protein molecular structure differences which may be induced by bioethanol processing. The protein molecular structure changes could be an important factor reflecting the effect of bioethanol processing on the nutritional value of DDGS. The applications of protein molecular structure detection in feed science are at a preliminary stage and a limited number of feedstuffs have been analyzed. DDGS is a valuable feedstuff which is produced through a series of physical and chemical processing steps. Investing protein molecular structure changes may contribute to the evaluation of the digestive characteristics of protein in DDGS. Currently, only a preliminary study with limited DDGS samples has been reported in the literature (Yu and Nuez-Ortín, 2010). Thus, a larger scale of protein molecular structure study is necessary for revealing the relationship between protein molecular structure and digestive characteristics of the proteins in DDGS.

4. Using Molecular Spectroscopy Techniques to Study Protein Structures and Their Relationship to Digestive Characteristics of DDGS

4.1. Introduction

Understanding the nutritional value, digestive behavior and nutrient supply of feeds to animals is vital for accurate ration formulation. Traditional wet chemical analysis, *in situ* animal trials and nutrient supply models have been widely used for investigating feed quality and animal nutrition (Ørskov and McDonald, 1979; AOAC, 1990; Tamminga et al., 1994; NRC, 2001). However, knowing the chemical composition of a feed does not fully explain its utilization, digestibility and net nutrient supply to animals. This is because traditional wet chemical analysis only considers total feed composition, but does not consider the feed's internal structure which is destroyed during chemical analysis. Recent studies show that protein molecular structure is related to the availability of nutrients in the gastrointestinal tract of the animal (Yu, 2005b, 2006a, 2010; Yu and Nuez-Ortín, 2010). It has been reported that changes in protein, lipid and carbohydrate structure detected by molecular spectroscopy can influence the nutrient value, utilization, availability, and digestive behavior of a feed (Yu, 2005b, 2006a, 2010; Yu and Nuez-Ortín, 2010).

Protein molecular structure can influence the utilization of proteins in various feedstuffs (Yu, 2005b; Doiron et al., 2009a; Yu et al., 2009; Liu and Yu, 2010a; Yu and Nuez-Ortín, 2010). However, for dried distillers grains with solubles (DDGS) which are high in fiber and protein, limited information is available on their protein structure (Yu and Nuez-Ortín, 2010). Distinguished from other plant based feed sources, DDGS is produced under a series of complex procedures including grinding, heating, fermenting, distillation and drying (Nichols and Bothast, 2008). Protein availability may change, even with similar protein content (Nuez-Ortín and Yu, 2009, 2010b). The higher acid detergent insoluble protein content may result in a decrease in protein availability to animals (Nuez-Ortín and Yu, 2010a). However, changes in the molecular structure of protein changes may also be one of the reasons that lead to the decreased protein availability.

To understand protein structure, scientists have utilized a number of spectroscopy techniques, including mid-infrared (Mid-IR), nuclear magnetic resonance and X-rays (Wuthrich,

1989; Kallen et al., 1991; Hering and Haris, 2009). These different types of spectroscopy have advantages and disadvantages. For example, X-ray crystallography could give the most detailed information regarding a single protein at an atomic level, but it is not possible to detect all proteins using this technique (Kong and Yu, 2007). Magnetic resonance spectroscopy can be used to investigate protein in solution (Kong and Yu, 2007). However, it is difficult to integrate the spectra of a large protein (Surewicz and Mantsch, 1988; Kong and Yu, 2007). Infrared spectroscopy has been used as a rapid, accurate, efficient analytical tool in various academic fields (Stuart, 2004). Fourier Transform Infrared Spectroscopy (FTIR) can be used to evaluate protein structure. The main limitation of infrared spectroscopy in studying protein secondary structure is the issue that you cannot quantify specific structures, but you can estimate relative protein structure. It is a good tool for comparing different samples and detecting small changes in protein molecular structure (Surewicz et al., 1993). Amide I and amide II bands are highly sensitive to protein secondary structure changes (Miller, 2002). Protein secondary structure mainly involves the α helix, β sheet and small amounts of β turn and random coils (Nelson and Cox, 2005). Fourier self deconvolution (FSD) and the second derivative analyses are used to identify α helix and β sheet peaks under the amide I band.

The hypotheses of this study are that bioethanol processing changes protein molecular structure of DDGS compared with the original cereal grain and that the induced protein structural changes caused by processing can be detected by molecular spectroscopy techniques [Synchrotron Based Fourier Transform Infrared Microspectroscopy (SFTIRM) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)]. The objectives of this study were: 1) to identify protein molecular structures of different DDGS; 2) to identify differences in protein molecular structures between grains and their DDGS and between different DDGS using two molecular spectroscopy techniques namely SFTIRM and DRIFT; 3) to quantify changes in protein structure in relation to the digestive characteristics of the protein in dairy cattle and to determine the most important structural features for DDGS and establish prediction equations to estimate digestive characteristics.

4.2. Materials and Methods

4.2.1. Feeds utilized

The three batchs of triticale grain and the three batches of triticale DDGS used for protein structure analysis were the same as used in Chapter 3. In addition, 17 wheat DDGS, corn DDGS, blend DDGS (wheat: corn = 70:30), wheat and corn samples described by Nuez-Ortín and Yu (2009) were also included in this protein molecular structure study. For these samples, the protein digestive characteristics have been previously described (Nuez-Ortín and Yu, 2009, 2010a). In this study, a comprehensive correlation and a multiple regression analysis was conducted.

4.2.2. Sample preparation for DRIFT spectroscopy

All samples were ground through 0.25 mm screen twice with a Retsch Grinder ZM100 (Brinkmann Instruments Ltd, Mississauga, ON) and then mixed in a 2 ml centrifuge tube with potassium bromide powder in a ratio of 1: 4 and vortexed for 1 min.

4.2.3. DRIFT molecular spectroscopy data collection and analysis

DRIFT molecular spectroscopy was performed using a Bio-Rad FTS-40 with a ceramic infrared source and MCT detector (Bio-Rad Laboratories, Hercules, CA) at the Saskatchewan Structural Sciences Center (SSSC, Saskatoon, SK). Each feed sample was scanned five times. Data was collected using Win-IR software installed in the coupled computer system. Spectra were generated from the Mid-IR (ca. 4000-800 cm^{-1}) portion of the electromagnetic spectrum with 256 scans co-added and a spectral resolution of 4 cm^{-1}. The collection of background spectra (potassium bromide powder) was performed prior to the sample spectra collection using the same settings. Spectral analysis was conducted using OMNIC 7.3 Software (Thermo Nicolet, Madison, WI). Baseline correction was done for all spectra prior to further interpretation.

4.2.4. Sample preparation for synchrotron based SFTIR microspectroscopy

To determine the protein molecular structures of the original cereal grains with SFTIRM, a total of nine batches of cereal grains (three batches of triticale, three batches of wheat and three batches of corn) were used for spectra collection. Five seeds for each batch of cereal grain (total of 45 seeds) were randomly selected, processed using an ASP-300s automated vacuum tissue processor (Leica Microsystems, Wetzlar, HE) and cut into thin cross sections (6 μm thickness) by a microtome. The unstained cross sections of the tissues were immediately transferred onto barium fluoride windows (1 mm thickness, 13 mm diameter, Spectral Systems, Hopewell Junction, NY) for synchrotron FTIR microspectroscopic work in transmission mode.

4.2.5. Synchrotron based SFTIR microcpectroscopy data collection and analysis

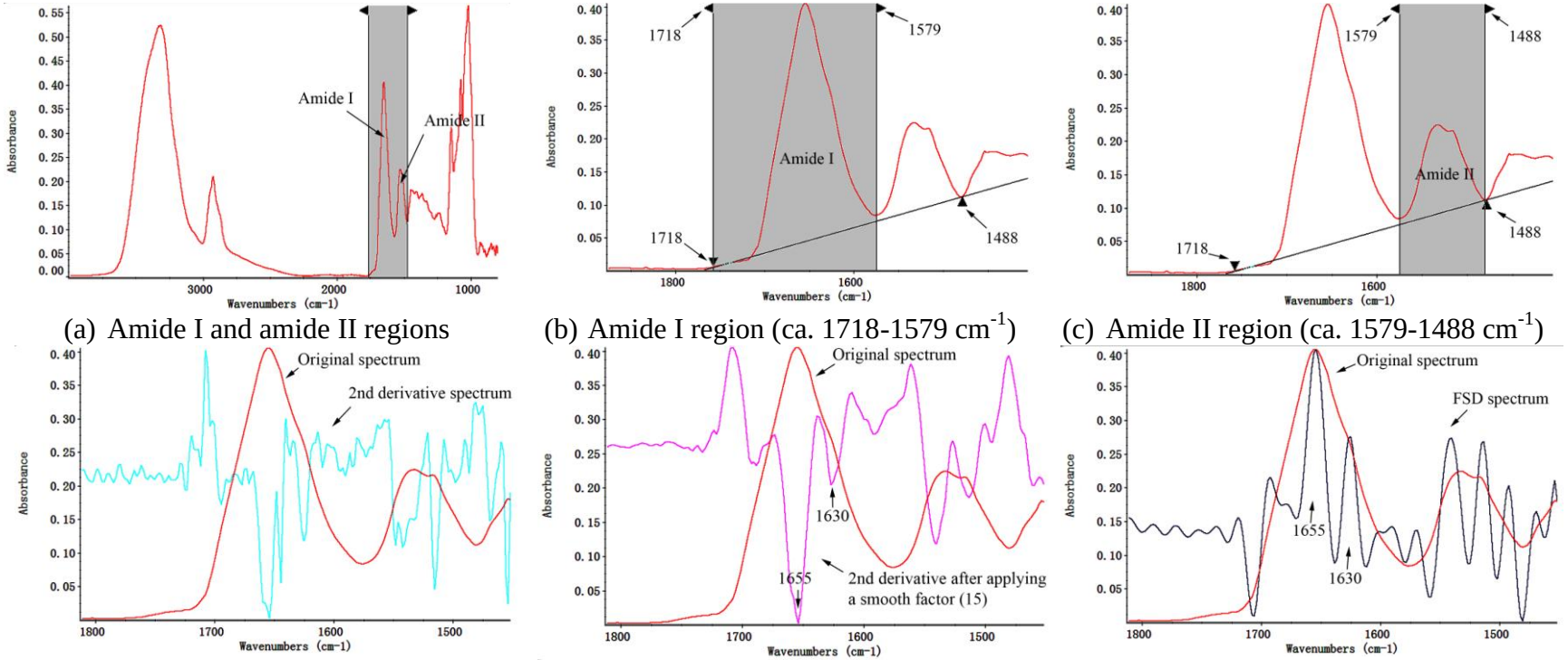
The SFTIRM spectra collection was performed using a Thermo Nicolet Magna 860 Step-Scan FTIR (Thermo Fisher Scientific, Waltham, MA) Spectrometer equipped with a Spectra Tech Continuum IR Microscope (Spectra-Tech, Shelton, CT) and liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. The infrared microspectroscopy instrument was configured with a synchrotron light beamline from U2B station, Brookhaven National Laboratory National Synchrotron Light Source, U.S. Department of Energy (NSLS-BNL, Upton, NY).

A range of 30 to 50 spot samples for each seed were randomly scanned in the relatively pure protein area in the endosperm region between 100-600 μm from the epidermis. The spectra were collected in the Mid-IR range (ca. 4000-800 cm^{-1}) at a resolution of 4 cm^{-1} with 128 scans co-added on each spot. The aperture size setting was adjusted to 10 × 10 μm. Background spectra were collected prior to the sample spectra collection. Scanned visible images were obtained using a charge-coupled device camera (CCD) linked to the infrared images. Nicolet OMNIC software 7.3 (Spectra Tech, Madison, WI) was used to collect and analyze spectra. Univariate and multivariate analysis were performed following the spectra collection.

4.2.6. Univariate spectral analysis

Protein molecular structure is usually determined from two primary bands in the spectra namely the amide I and amide II region (ca. 1720-1485 cm^{-1}). The amide I contains 80% C=O stretching, 10% C-N and 10% N-H (Jackson and Mantsch, 1991; Stuart, 2004) and was

identified in this study in the range of ca. 1718-1579 cm^{-1}. The amide II consists of 40% C-N stretching and 60% N-H bending vibrations and was found in the range of ca. 1579-1488 cm^{-1}. Both amide I and amide II are used in protein molecular structure studies, although compared with amide I, amide II is less useful because of the involvement of multiple functional groups which lead to complex vibrations. The amide I and amide II peak area absorption intensity and their ratio were calculated. With Fourier self-deconvolution (FSD) (Kauppinen et al., 1981; Griffiths and Pariente, 1986) or the second derivative functions in the OMNIC software, amide I was further resolved into several multi-component peaks where α-helix (center at ca. 1655 cm^{-1}) and β-sheet (center at ca. 1630 cm^{-1}) were identified (Figure 4.1). The intensity of the peak height of the α-helix and β-sheet and their ratio were also calculated according to Yu (2006b).



(a) Amide I and amide II regions (b) Amide I region (ca. 1718-1579 cm^{-1}) (c) Amide II region (ca. 1579-1488 cm^{-1})

(d) 2nd derivative spectrum (e) 2nd derivative spectrum after applying a smooth factor (15) (f) FSD spectrum

Figure 4.1 A typical Synchrotron Based Fourier Transform Infrared Microspectroscopy spectrum and its 2nd derivative and Fourier self-deconvolution spectra for triticale in the amide I and amide II regions (ca. 1718-1488 cm^{-1})

4.2.7. Multivariate spectral analysis

Multivariate spectra analysis performed included Agglomerative Hierarchical Cluster Analysis (CLA) and Principal Component Analysis (PCA). These analyses were performed using Statistica 8 Software (StatSoft Inc, Tulsa, OK). For Agglomerative Hierarchical Cluster Analysis, Ward's Algorithm Method was used for clustering (Miller et al., 2000) and clusters were displayed as dendrograms (Cytospec, 2004). For Principal Component Analysis, the 1st principal (PC1) vs. the 2nd principal component (PC2) were generated as a scatter plot and total variances explained by PC1 and PC2 were calculated. The two multivariate spectral analyses were reviewed by Yu (2005c, a).

4.2.8. Statistical analysis

Statistical analyses were performed using the PROC MIXED procedure of SAS 9.1.3 (SAS Institute, Cary, NC). Significance was declared at $P < 0.05$, and trends at $P \leq 0.10$. Differences among the treatments were evaluated using Tukey's multiple comparison test (Tukey-Kramer for unequalized sample size).

The DRIFT spectroscopic data were analyzed with a Completely Randomized Design model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij (amide I, amide II, ratio of amide I to II, α-helix, β-sheet and ratio of α-helix to β-sheet); μ was the population mean for the variable; T_i was the effect of different cereal grains and DDGS, as a fixed effect, and e_{ij} was the random error associated with the observation ij.

The SFTIRM spectroscopic data were analyzed using a Completely Nested Design Model with block structure: $Y_{ijkl} = \mu + T_i + S(T)_j + R_k + e_{ijkl}$, where Y_{ijkl} was an observation of the dependent variable ijkl (amide I, amide II, ratio of amide I to II, α-helix, β-sheet and ratio of α-helix to β-sheet); μ was the population mean for the variable; T_i was the effect of the different cereal grains and DDGS, as a fixed effect; $S(T)_j$ was the seeds nested within treatment, as a random effect, R_k was two experimental run (two trips to NSLS Synchrotron Center) and e_{ijkl} was the random error associated with the observation ijkl. The detailed methodology was explained by Yu (2004).

Correlation between the changes in protein structure (amide I, amide II, ratio of amide I to II, α-helix, β-sheet and ratio of α-helix to β-sheet) and the changes in chemical composition, CNCPS protein fractions, *in situ* degradation kinetics, intestinal digestion and predicted nutrient supply to dairy cattle in DDGS and cereal grains were analyzed using the CORR procedure of SAS software (SAS Institute, 2003) with a nonparametric correlation method (Spearman) as some of the nutrient data were not normally distributed. Normality tests were performed using the UNIVARIATE procedure of SAS with Normal and PLOT options.

Multiple regression with variable selection analysis was carried out using the "PROC REG" procedure with a model as follows:

Model: Y = amide I (A_I) + amide II (A_II) + amide I to amide II ratio (R_I_II) + α-helix (H_1655) + β-sheet (H_1630) + α-helix to β-sheet ratio (R_α_β).

The model used a "STEPWISE" option with variable selection criteria: "SLENTRY = 0.05, SLSTAY = 0.05". All variables left in the final prediction models were significant at the 0.05 level. Residual analysis was performed using the UNIVARIATE procedure of SAS with Normal and PLOT options. Collinearity detection was conducted using the VIF option of SAS to eliminate the influence of correlated dependent variables to the prediction of independent variables.

4.3. Results and Discussion

4.3.1. Using DRIFT spectroscopy to characterize and compare protein structure profiles among different cereal grains and their DDGS

4.3.1.1. Quantifying protein molecular structure amide I to amide II ratio

Table 4.1 gives the protein molecular structure parameters of the cereal grains and DDGS samples. Compared with cereal grains, DDGS exhibited significant differences in their amide profiles ($P<0.01$). These differences may result from bioethanol processing. The decrease ($P<0.01$) in intensity of amide I to amide II ratio from the grains to their DDGS agreed with the results of Yu et al. (2010) (grain vs. DDGS = 4.58 vs. 2.84, $P<0.05$). By comparing each grain with its corresponding DDGS, it was found that all three grains were higher in the spectral

intensity of amide I and amide I to amide II ratio than their DDGS. Amide II did not change in a similar pattern, as it increased in intensity in DDGS with the exception of triticale DDGS. In terms of the amide I to amide II ratio, there were significant differences among wheat DDGS, corn DDGS and triticale DDGS, with blend DDGS having results similar to the wheat DDGS and triticale DDGS. A comparison among the different grains showed that triticale is significantly higher in amide I to amide II ratio than wheat and corn (5.70 vs. 4.91 and 5.70 vs. 4.95, $P<0.01$).

4.3.1.2. Quantifying protein molecular structure α helix to β sheet ratio

Table 4.1 also shows the protein molecular structure characteristics in terms of α helix to β sheet ratio. Grains showed significantly different results in α helix, β sheet and their ratio compared with DDGS. The intensity of α helix and β sheet height was higher in all three grains (wheat, corn and triticale) than their DDGS (wheat DDGS, corn DDGS and triticale DDGS). However, in terms of α helix to β sheet ratio, only corn and corn DDGS showed a significant decrease (1.38 vs. 1.21, $P<0.01$). This result disagrees with the results of Yu et al. (2010) who showed increases from the original grain to DDGS in α helix and β sheet heights. Doiron et al. (2009a) reported that heating Vimy flaxseed at 120°C for 40 and 60 min increased the α helix to β sheet ratio, which is opposite to the results of the current study and Yu et al. (2010). The discrepancy might arise from differences in heating conditions, because bioethanol processing requires a series of heating procedures such as cooking (non-pressurized or pressurized) and drying under different temperatures. The inconsistency in the intensity of α helix, β sheet heights may be caused by the shift of spectra. When investigating protein molecular structure using infrared spectroscopy, the identifications of different bands are usually based on the shape of peaks. However, with different samples or different experimental environments, a shift of spectra may happen. This may lead to a shift of the peak center, therefore peak height may differ accordingly.

Table 4.1 Comparison of different cereal grains with their dried distillers grains with solubles (DDGS) in terms of protein molecular structure spectral profiles using Diffuse Reflectance Infrared Fourier Transform Spectroscopy

	Feed sources									
	Wheat (n=3)	Corn (n=3)	Triticale (n=3)	Wheat DDGS (n=5)	Corn DDGS (n=3)	Triticale DDGS (n=3)	Blend* DDGS (n=3)	SEM	P Values	Grains vs. DDGS P values
Protein molecular structure spectral profiles (Unit: Absorbance)										
Amide I area	19.21^{b}	13.56^{c}	21.17^{a}	11.30^{d}	8.48^{e}	6.18^{f}	9.04^{e}	0.399	<0.01	<0.01
Amide II area	3.94^{c}	2.74^{d}	3.74^{c}	5.53^{b}	6.54^{a}	3.57^{c}	5.14^{b}	0.139	<0.01	<0.01
Amide I to Amide II ratio	4.91^{b}	4.95^{b}	5.70^{a}	2.03^{c}	1.29^{e}	1.73^{d}	1.75^{cd}	0.075	<0.01	<0.01
α Helix height	0.26^{a}	0.21^{b}	0.28^{a}	0.14^{c}	0.12^{d}	0.08^{e}	0.11^{d}	0.005	<0.01	<0.01
β Sheet height	0.21^{b}	0.15^{c}	0.23^{a}	0.10^{d}	0.10^{de}	0.07^{f}	0.09^{ef}	0.004	<0.01	<0.01
α Helix to β sheet ratio	1.26^{bc}	1.38^{a}	1.21^{c}	1.31^{b}	1.21^{c}	1.20^{c}	1.26^{bc}	0.018	<0.01	<0.01

SEM = standard error of mean.

$^{a-f}$Means with different superscripts in the same row are significantly different (P<0.05).

Multi-treatment comparison method: Tukey-Kramer

*Blend DDGS produced from a blend of 70% wheat and 30% corn

4.3.2. Using synchrotron based FTIR microspectroscopy to characterize and compare protein spectral profiles in the endosperm regions of different cereal grains

4.3.2.1. Quantifying protein molecular structure amide I to amide II ratio and protein secondary structure

The synchrotron based SFTIRM was used to detect protein structure spectral profiles in the seed endosperm which is the protein rich area of cereal grains (Halford and Shewry, 2007) (Table 4.2). This information is an important supplement for DRIFT spectra collection, because it identifies the relationship of intact seed with ground, powdered cereal grains. In terms of amide profile, triticale was significantly higher than corn and wheat in amide I and amide II area, while lower than wheat in amide I to amide II ratio. In terms of α helix and β sheet and their ratio, triticale showed the greatest values among the three cereal grains in α helix and β sheet intensities, but had the lowest value for α helix to β sheet ratio. Corn had the highest α helix to β sheet ratio among the three cereal grains, which is consistent with the data collected by DRIFT. However, considering that there is also some inconsistency between the data collected using the two techniques, a comparison and correlation study is needed to reveal the relationship between the two techniques.

4.3.2.2. Comparison and correlation of DRIFT and SFTIRM data

The paired t test and Pearson correlation study was carried out to reveal the relationship of DRIFT and SFTIRM data in determining protein structure spectral profiles of the three types of cereal grains (Table 4.3). Significant differences were found in amide II area intensity ($P<0.05$), amide I to amide II ratio ($P<0.05$) and α helix to β sheet ratio ($P<0.01$) between the two techniques. These results indicate that depending on the different samples and regions (ground whole seed sample for DRIFT vs. seed endosperm region for SFTIRM), results can be greatly affected. While some values detected by the two techniques were significantly different, the amide I to amide II ratio ($P<0.05$) and α helix to β sheet ratio ($P<0.01$) were highly correlated between the two techniques. In amide I to amide II ratio, the DRIFT technique was negatively correlated to SFTIRM ($R=-0.68$, $P<0.05$). For the α helix to β sheet ratio, this correlation was positive ($R=0.88$, $P<0.01$). This indicates that the protein structure in the endosperm region of

the three cereal grains was highly associated with overall protein structure in the ground samples. However, the different results obtained by the two techniques indicate the need to examine protein structure in different sample states and different regions. Also, the protein molecular structure in different structure regions (e.g. aleuronic layer, endosperm and embryo) should be studied in order to provide information for new variety breeding or quality monitoring of seeds. For this type of research, only synchrotron based techniques are applicable.

Table 4.2 Comparison between different cereal grains at the endosperm region in terms of protein molecular structure spectral profiles using Synchrotron Based Fourier Transformed Infrared Microspectroscopy

	Endosperm region			SEM	P values
	Wheat (n=3)	Corn (n=3)	Triticale (n=3)		
Protein molecular structure spectral profiles (Unit: Absorbance)					
Amide I area	14.83[b]	16.17[b]	21.45[a]	2.562	<0.01
Amide II area	3.46[b]	4.30[b]	5.95[a]	1.184	<0.01
Amide I to Amide II ratio	4.64[a]	4.20[b]	4.17[b]	0.619	<0.05
α Helix height	0.21[b]	0.25[ab]	0.29[a]	0.039	<0.01
β Sheet height	0.15[b]	0.16[b]	0.23[a]	0.020	<0.01
α Helix to β sheet ratio	1.41[b]	1.54[a]	1.33[c]	0.123	<0.01

SEM = standard error of mean.

[a-c]Means with different superscripts in the same row are significantly different (P<0.05).

Multi-treatment comparison method: Tukey

Table 4.3 Comparison between the Diffuse Reflectance Infrared Fourier Transform Spectroscopy approach and the Synchrotron Based Fourier Transform Infrared Microspectroscopy approach in the determination of protein molecular structure spectral profiles of three cereal grains (wheat, corn and triticale) using paired t test and Pearson correlation

	Comparison DRIFT vs. SFTIRM approach					Correlation analysis DRIFT vs. SFTIRM approach	
	$Mean^{DRIFT}$	$Mean^{SFTIRM}$	Difference	SED	P values	R	P values
Protein molecular structure spectral profiles (Unit: Absorbance)							
Amide I area	17.98	17.28	0.70	1.118	0.55	0.47	0.21
Amide II area	3.47	4.51	-1.03	0.419	<0.05	-0.09	0.81
Amide I to Amide II ratio	5.18	4.34	0.85	0.259	<0.05	-0.68	<0.05
α Helix height	0.25	0.25	0.00	0.015	0.89	0.20	0.61
β Sheet height	0.20	0.18	0.02	0.011	0.13	0.57	0.11
α Helix to β sheet ratio	1.29	1.43	-0.14	0.017	<0.01	0.88	<0.01

SED= standard error of the difference. R = Pearson correlation coefficient.

4.3.3. Correlations between protein structure spectral parameters and metabolizable protein characteristics

To relate protein molecular structure to protein profiles, CNCPS subfractions, *in situ* rumen undegraded protein, intestinal RUP degradability and nutrient supply prediction for dairy cattle, a correlation study was conducted (Table 4.4 to 4.5). Since some of the data were not normally distributed, a Spearman (rank) correlation was conducted. Spectral data obtained by both DRIFT and SFTIRM techniques were included in the correlation study.

4.3.3.1. Amide I and amide II profiles in relation to CNCPS protein subfractions, *in situ* rumen undegraded protein, estimated intestinal RUP degradability and predicted nutrient supply to dairy cattle

The amide I to amide II ratio is influenced by cereal grain variety (Yu, 2007), autoclave processing (Doiron et al., 2009a), and gene transformation (Yu et al., 2009). Thus, it is reasonable to assume that bioethanol processing which includes a series of processing procedures including fermentation, distillation and dry heating, is also related to the amide I to amide II ratio. For protein profiles, crude protein was found to be negatively correlated to the amide I to amide II ratio ($R=-0.65$, $P<0.01$). Negative correlations were also found between the amide I to amide II ratio between neutral detergent insoluble crude protein ($R=-0.67$, $P<0.01$) and acid detergent insoluble crude protein ($R=-0.68$, $P<0.01$). In contrast, a positive correlation was found between soluble crude protein and the amide I to amide II ratio with a $R=0.75$ ($P<0.01$). The results indicate that a higher amide I to amide II ratio is associated with a higher soluble crude protein in DDGS and cereal grains. For total digestible crude protein, the results showed that the amide I to amide II ratio had a modest negative correlation ($P<0.01$) with a $R=-0.65$. For the Cornell Net Carbohydrate and Protein System (CNCPS) protein subfractions, the protein amide I to amide II ratio had a positive correlation to the protein PB1 subfraction ($P<0.01$) with a $R=0.78$, but a negative correlation to PB3 ($R=-0.67$, $P<0.01$) and PC ($R=-0.68$, $P<0.01$). However, there was no correlation with PA, PB2 and true protein (the sum of PB1, PB2 and PB3).

For protein degradation kinetics, the results showed that the protein amide I to amide II ratio had a negative correlation ($R=-0.59$, $P<0.01$) to RUP (% CP). Since RUP^{DVE} (g/kg DM) and RUP^{NRC} (g/kg DM) were both calculated from RUP (% CP) with a different multiple factor

(1.11 vs. 1.00), there were identical negative correlations between them and the amide I to amide II ratio. The results indicate that a lower amide I to amide II ratio was associated with a higher RUP value in both cereal grains and DDGS.

For the prediction of protein supply to dairy cattle, there were correlations between truly absorbable intestinal protein (abbreviated as DVE in the DVE/OEB system and MP in NRC-2001 model) and degraded protein balance (abbreviated as OEB in the DVE/OEB system and DPB^{NRC} in NRC-2001 model) and the amide I to amide II ratio (Table 4.5). The results showed that the protein amide I to amide II ratio was negatively correlated to the DVE value (R=-0.77, P<0.01) and the OEB value (R=-0.65, P<0.01). Although calculation equations differ between the DVE/OEB system and the NRC-2001 model, the protein amide I to amide II ratio was negatively correlated to the MP value (R=-0.73, P<0.01) and the DPB^{NRC} value (R=-0.50, P<0.01). A previous study targeting only DDGS, gave different results on OEB (R=0.97, P<0.05) in relation to amide I to amide II ratio (Yu and Nuez-Ortín, 2010). A possible reason could be the inclusion of cereal grains and the application of SFTIRM technique in the present study.

4.3.3.2. Protein secondary structure profiles (α helix, β sheet and their ratio) in relation to protein profiles, CNCPS protein subfractions, *in situ* rumen undegraded protein, estimated intestinal RUP degradability and predicted nutrient supply to dairy cattle

The α helix to β sheet ratio was found to be weakly and negatively correlated to crude protein content (R=-0.42, P<0.05). Weak negative correlations were also found between the α helix to β sheet ratio and neutral detergent insoluble crude protein (R=-0.44, P<0.05), acid detergent insoluble crude protein (R=-0.58, P<0.01) and total digestible protein (R=-0.43, P<0.05). In contrast, a weak positive correlation was found between soluble crude protein and α helix to β sheet ratio with a low R=0.35 (P<0.05). The results indicate that a higher α helix to β sheet ratio is associated with a higher soluble crude protein in DDGS and cereal grains. For the Cornell Net Carbohydrate and Protein System (CNCPS) protein subfractions, the α helix to β sheet ratio had a positive correlation to the protein PB1 subfraction (P<0.05) with a R=0.44, but a negative correlation to PB3 (R=-0.45 P<0.01) and PC (R=-0.58, P<0.01). However, it has no correlation with PA, PB2 and true protein (the sum of PB1, PB2 and PB3).

For protein degradation kinetics, the results showed that the α helix to β sheet ratio had no correlation to RUP (%CP) ($P>0.05$). A previous study (Yu and Nuez-Ortín, 2010) reported a negative correlation between intestinal digestibility of RUP *in vitro* to the α helix to β sheet ratio ($R=-0.95$, $P<0.05$). In the present study, this correlation also tended to be negative ($R=-0.35$, $P=0.05$). These results indicate that a higher α helix to β sheet ratio may lead to lower intestinal protein availability. These results differ from the previous studies that suggested that a higher β sheet content may cause lower nutrient availability (Yu, 2005b). Different heating methods (autoclaving vs. dry) may account for the changes (Doiron, 2008; Yu and Nuez-Ortín, 2010). For modeling protein supply prediction to dairy cattle, the α helix to β sheet ratio had a weak negative correlation with OEB ($R=-0.49$, $P<0.01$), MP ($R=-0.36$, $P<0.01$), and DPB^{NRC} ($R=-0.42$, $P<0.01$).

Table 4.4 Correlation between protein molecular structure spectral profiles and protein profiles, protein subfractions (Cornell Net Carbohydrate and Protein System) and protein rumen degradation kinetics in different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS) using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy

	Amide I		Amide II		R_I_II		α helix		β sheet		R_α_β	
	R	P values	R	P values	R	P values	R	P values	R	P values	R	P values
Protein profiles												
Crude protein (% DM)	-0.56	<0.01	0.56	<0.01	-0.65	<0.01	-0.66	<0.01	-0.64	<0.01	-0.42	<0.05
Soluble crude protein (% CP)	0.65	<0.01	-0.51	<0.01	0.75	<0.01	0.66	<0.01	0.66	<0.01	0.35	<0.05
NDICP (% CP)	-0.60	<0.01	0.47	<0.01	-0.67	<0.01	-0.70	<0.01	-0.67	<0.01	-0.44	<0.05
ADICP (% CP)	-0.57	<0.01	0.44	<0.05	-0.68	<0.01	-0.59	<0.01	-0.59	<0.01	-0.58	<0.01
Total digestible CP (% DM)	-0.56	<0.01	0.55	<0.01	-0.65	<0.01	-0.67	<0.01	-0.64	<0.01	-0.43	<0.05
Protein fractions												
PA (% CP)	-0.15	0.40	-0.50	<0.01	0.17	0.35	-0.15	0.43	-0.15	0.40	0.28	0.12
PB1 (% CP)	0.75	<0.01	-0.45	<0.05	0.78	<0.01	0.79	<0.01	0.78	<0.01	0.44	<0.05
PB2 (% CP)	0.40	<0.05	-0.13	0.48	0.33	0.07	0.42	<0.05	0.44	<0.05	-0.02	0.91
PB3 (% CP)	-0.60	<0.01	0.49	<0.01	-0.67	<0.01	-0.69	<0.01	-0.67	<0.01	-0.45	<0.01
PC (% CP)	-0.57	<0.01	0.44	<0.05	-0.68	<0.01	-0.59	<0.01	-0.59	<0.01	-0.58	<0.01
True protein (% CP)	0.45	<0.05	0.23	0.21	0.21	0.26	0.45	<0.05	0.45	<0.05	0.00	1.00
Protein rumen degradation kinetics												
Rumen undegraded protein (% CP)	-0.62	<0.01	0.33	0.07	-0.59	<0.01	-0.54	<0.01	-0.59	<0.01	0.09	0.63
EDCP (% CP)	0.62	<0.01	-0.33	0.07	0.59	<0.01	0.54	<0.01	0.59	<0.01	-0.09	0.63
RUP^{DVE} (g/kg DM)	-0.80	<0.01	0.43	<0.05	-0.76	<0.01	-0.80	<0.01	-0.82	<0.01	-0.13	0.49
RUP^{NRC} (g/kg DM)	-0.80	<0.01	0.43	<0.05	-0.76	<0.01	-0.80	<0.01	-0.82	<0.01	-0.13	0.49
EDCP (g/kg DM)	-0.41	<0.05	0.26	0.15	-0.42	<0.05	-0.52	<0.01	-0.48	<0.01	-0.45	<0.05

Note: R_I_II means amide I to amide II intensity ratio; R_α_β means α helix to β sheet intensity ratio.
Correlation coefficient (R) was calculated using Spearman method (Ranking correlation).
Abbreviations: NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; PA, non-protein nitrogen; PB1, rapidly degradable protein fraction; PB2, intermediately degradable protein fraction; PB3, slowly degradable protein fraction; PC, unavailable protein fraction; RUP^{DVE} (RUP^{NRC}), rumen undegraded feed protein (in DVE/OEB or NRC-2001 models); EDCP, effectively degradable feed protein.

Table 4.5 Correlation between protein molecular structure spectral profiles and predicted nutrient supply using the DVE/OEB 1994 and NRC-2001 models in different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS) using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy

	Amide I		Amide II		R_I_II		α helix		β sheet		R_α_β	
	R	P values	R	P values	R	P values	R	P values	R	P values	R	P values
Modeling protein nutrients in the DVE/OEB system												
FOM (g/kg DM)	0.59	<0.01	-0.25	0.17	0.55	<0.01	0.50	<0.01	0.57	<0.01	-0.09	0.64
$AMCP^{DVE}$ (g/kg DM)	0.59	<0.01	-0.25	0.17	0.55	<0.01	0.50	<0.01	0.57	<0.01	-0.09	0.64
ENDP (g/kg DM)	-0.44	<0.05	0.16	0.39	-0.43	<0.05	-0.54	<0.01	-0.50	<0.01	-0.50	<0.01
RUP^{DVE} (g/kg DM)	-0.80	<0.01	0.43	<0.01	-0.76	<0.01	-0.80	<0.01	-0.82	<0.01	-0.13	0.49
dRUP (%RUP)	-0.26	0.15	0.55	<0.01	-0.44	<0.05	-0.34	0.06	-0.31	0.08	-0.35	0.05
$ARUP^{DVE}$ (g/kg DM)	-0.79	<0.01	0.44	<0.01	-0.77	<0.01	-0.80	<0.01	-0.83	<0.01	-0.16	0.39
DVE (g/kg DM)	-0.76	<0.01	0.46	<0.01	-0.77	<0.01	-0.79	<0.01	-0.80	<0.01	-0.21	0.24
OEB (g/kg DM)	-0.62	<0.01	0.39	<0.05	-0.65	<0.01	-0.71	<0.01	-0.69	<0.01	-0.49	<0.01
Modeling protein nutrients in the NRC-2001 model												
$AMCP^{NRC}$ (g/kg DM)	-0.23	0.21	0.20	0.28	-0.26	0.15	-0.30	0.09	-0.24	0.19	-0.31	0.08
$ARUP^{NRC}$ (g/kg DM)	-0.79	<0.01	0.44	<0.05	-0.77	<0.01	-0.80	<0.01	-0.83	<0.01	-0.16	0.39
AECP (g/kg DM)	-0.36	<0.05	0.61	<0.01	-0.53	<0.01	-0.44	<0.05	-0.41	<0.05	-0.34	0.06
MP (g/kg DM)	-0.68	<0.01	0.50	<0.01	-0.73	<0.01	-0.74	<0.01	-0.73	<0.01	-0.36	<0.05
DPB^{NRC} (g/kg DM)	-0.50	<0.01	0.27	0.14	-0.50	<0.01	-0.61	<0.01	-0.58	<0.01	-0.42	<0.05

Note: R_I_II means amide I to amide II intensity ratio; R_α_β means α helix to β sheet intensity ratio.
Correlation coefficient (R) was calculated using Spearman method (Ranking correlation).
Abbreviations: FOM, organic matter fermented in the rumen; $AMCP^{DVE}$, truly absorbed microbial protein in the small intestine; ENDP, endogenous protein in the small intestine; RUP^{DVE}, rumen undegraded feed protein estimated from the DVE/OEB 1994 model; dRUP, estimated intestinal digestibility of RUP; $ARUP^{DVE}$, truly absorbed rumen undegraded protein in the small intestine; DVE, truly digested protein in the small intestine; OEB, degraded protein balance; $AMCP^{NRC}$, truly absorbed microbial protein in the small intestine; $ARUP^{NRC}$, truly absorbed rumen undegraded protein in the small intestine; AECP, truly absorbed endogenous protein in the small intestine; MP, metabolizable protein; DPB^{NRC}, degraded protein balance.

Table 4.6 Multiple regression with variable selection analysis to find the most important variables to predict nutrient supply using protein molecular structure spectral parameters[1] (A_I, A_II, R_I_II, H_1655, H_1630, R_α_β) collected using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy in different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS), Part I

Predicted variables (Y)	Variables left in the model with P<0.05	Prediction Equations Y= a + b1 × x1 + b2 × x2 + ...	R^2 values	RSD	P values
Protein profiles					
Crude protein (%DM)	R_I_II left in the model	CP=45.58-6.67×R_I_II	0.79	5.52	<0.01
Non-protein N (%SCP)	H_1655 left in the model	NPN=133.18-310.11×H_1655	0.47	24.93	<0.01
SCP (%CP)	R_I_II, R_α_β left in the model	SCP=-67.40+4.73×R_I_II+60.12×R_α_β	0.41	14.27	<0.01
ADICP (%CP)	H_1655 left in the model	ADICP=9,79-37.05×H_1655	0.44	3.11	<0.01
NDICP (%CP)	R_I_II left in the model	NDICP=63.52-10.76×R_I_II	0.72	10.77	<0.01
Protein fractions					
PA (%CP)	A_II, H_1630 left in the model	PA=32.49-2.42×A_II-37.30×H_1630	0.38	4.33	<0.01
PB1 (%CP)	R_I_II left in the model	PB1=-9.26+6.08×R_I_II	0.29	15.58	<0.01
PB2 (%CP)	H_1630 left in the model	PB2=23.68+152.70×H_1630	0.26	14.76	<0.01
PB3 (%CP)	R_I_II left in the model	PB3=54.80-9.04×R_I_II	0.61	11.68	<0.01
PC (%CP)	H_1655 left in the model	PC=9.79-37.05×H_1655	0.45	3.11	<0.01
True protein (%CP)	A_I, A_II left in the model	TP=60.38+0.87×A_I+1.90×A_II	0.48	5.16	<0.01
Protein digestible fractions					
tdCP (%DM)	R_I_II left in the model	tdCP=44.41-6.43×R_I_II	0.78	5.52	<0.01
TDN_{1X} (%)	H_1630 left in the model	TDN_{1X}=78.15+33.96×H_1630	0.15	4.62	<0.05

RSD= Residual standard deviation.

[1] Protein molecular structure spectral profiles (Unit: infrared absorbance intensity): A_I = peaks area intensity at ca. 1718-1579 cm^{-1}; A_II = peaks area intensity at ca. 1579-1488 cm^{-1}; R_I_II = amide I to amide II ratio; H_1655 = α helix peak height intensity, center at ca. 1655 cm^{-1}; H_1630 = β sheet peak height intensity, center at ca. 1630 cm^{-1}; R_α_β = α helix to β sheet ratio.

Abbreviations: SCP, soluble crude protein; ADICP, acid detergent insoluble crude protein; NDICP, neutral detergent insoluble crude protein; PA, non-protein nitrogen; PB1, rapidly degradable protein fraction; PB2, intermediately degradable protein fraction; PB3, slowly degradable protein fraction; PC, unavailable protein fraction; tdCP, total digestible crude protein; TDN_{1X}, total digestible nutrients at maintenance.

Table 4.7 Multiple regression with variable selection analysis to find the most important variables to predict nutrient supply using protein molecular structure spectral parameters[1] (A_I, A_II, R_I_II, H_1655, H_1630, R_α_β) collected using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy in different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS), Part II

Predicted variables (Y)	Variables left in the model with P<0.05	Prediction Equations Y= a + b1 × x1 + b2 × x2 + ...	R^2 values	RSD	P values
Protein rumen degradation kinetics					
S (%CP)	H_1655 left in the model	S=12.55-29.17×H_1655	0.15	5.21	<0.05
D (%CP)	H_1630, R_α_β left in the model	D=19.55+63.83×H_1630+44.02×R_α_β	0.44	7.46	<0.01
U (%CP)	R_α_β left in the model	U=52.92-35.57×R_α_β	0.29	6.03	<0.01
T0 (h)	No variables met the 0.05 significant level for entry the model				
K_d	A_I, R_α_β left in the model	K_d=25.94+0.98×A_I-22.76×R_α_β	0.55	4.66	<0.01
RUP (%CP)	A_I, A_II, R_α_β left in the model	RUP=-20.36-2.25×A_I+3.84×A_II+61.21×R_α_β	0.59	11.22	<0.01
EDCP (%CP)	H_1630, R_α_β left in the model	EDCP=120.36+2.25×A_I-3.84×A_II-61.21×R_α_β	0.59	11.22	<0.01
RUP^{DVE} (g/kg DM)	R_I_II left in the model	RUP^{DVE}=314.32-52.56×R_I_II	0.77	46.35	<0.01
RUP^{NRC} (g/kg DM)	R_I_II left in the model	RUP^{NRC}=283.17-47.35×R_I_II	0.77	41.75	<0.01
EDCP (g/kg DM)	R_I_II, R_α_β left in the model	EDCP=388.85-14.96×R_I_II-165.16×R_α_β	0.46	39.40	<0.01

RSD= Residual standard deviation.

[1] Protein molecular structure spectral profiles (Unit: infrared absorbance intensity): A_I = peaks area intensity at ca. 1718-1579 cm^{-1}; A_II = peaks area intensity at ca. 1579-1488 cm^{-1}; R_I_II = amide I to amide II ratio; H_1655 = α helix peak height intensity, center at ca. 1655 cm^{-1}; H_1630 = β sheet peak height intensity, center at ca. 1630 cm^{-1}; R_α_β = α helix to β sheet ratio.

Abbreviations: S, soluble fraction in the *in situ* incubation; D, insoluble, but potentially degradable fraction in the *in situ* incubation; U, undegradable fraction in the *in situ* incubation; T0, lag time; K_d, degradation rate; RUP, rumen undegraded feed protein; RUP^{DVE} (RUP^{NRC}), rumen undegraded feed protein (in DVE/OEB or NRC-2001 models); EDCP, effectively degradable feed protein.

Table 4.8 Multiple regression with variable selection analysis to find the most important variables to predict nutrient supply using protein molecular structure spectral parameters[1] (A_I, A_II, R_I_II, H_1655, H_1630, R_α_β) collected using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy in different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS), Part III

Predicted variables (Y)	Variables left in the model with P<0.05	Prediction Equations Y= a + b1 × x1 + b2 × x2 + ...	R^2 values	RSD	P values
Predicted nutrient supply using the DVE/OEB system					
FOM (g/kg DM)	A_I, R_α_β left in the model	FOM=816.23+16.44×A_I-302.00×R_α_β	0.56	74.80	<0.01
$AMCP^{DVE}$ (g/kg DM)	A_I, R_α_β left in the model	$AMCP^{DVE}$=78.06+1.57×A_I-28.88×R_α_β	0.56	7.15	<0.01
ENDP (g/kg DM)	H_1655, R_α_β left in the model	ENDP=21.50-18.23×H_1655-9.71×R_α_β	0.54	1.93	<0.01
dRUP (%RUP)	R_I_II left in the model	dRUP=0.87-0.03×R_I_II	0.23	0.08	<0.01
$ARUP^{DVE}$ (g/kg DM)	R_I_II left in the model	$ARUP^{DVE}$=257.42-44.19×R_I_II	0.75	41.45	<0.01
DVE (g/kg DM)	R_I_II left in the model	DVE=296.17-38.98×R_I_II	0.72	39.80	<0.01
OEB (g/kg DM)	R_I_II left in the model	OEB=86.98-21.58×R_I_II	0.53	32.67	<0.01
Predicted nutrient supply using the NRC-2001 model					
$AMCP^{NRC}$ (g/kg DM)	R_α_β left in the model	$AMCP^{NRC}$=156.56-79.14×R_α_β	0.38	10.91	<0.01
$ARUP^{NRC}$ (g/kg DM)	R_I_II left in the model	$ARUP^{NRC}$=231.91-39.81×R_I_II	0.75	37.34	<0.01
AECP (g/kg DM)	R_I_II left in the model	AECP=4.42-0.03×R_I_II	0.33	0.08	<0.01
MP (g/kg DM)	R_I_II left in the model	MP=300.96-43.32×R_I_II	0.76	39.85	<0.01
DPB^{NRC} (g/kg DM)	R_I_II, R_α_β left in the model	DPB^{NRC}=289.43-17.94×R_I_II-168.95×R_α_β	0.47	42.74	<0.01

RSD= Residual standard deviation.

[1] Protein molecular structure spectral profiles (Unit: infrared absorbance intensity): A_I = peaks area intensity at ca. 1718-1579 cm^{-1}; A_II = peaks area intensity at ca. 1579-1488 cm^{-1}; R_I_II = amide I to amide II ratio; H_1655 = α helix peak height intensity, center at ca. 1655 cm^{-1}; H_1630 = β sheet peak height intensity, center at ca. 1630 cm^{-1}; R_α_β = α helix to β sheet ratio.

Abbreviations: FOM, organic matter fermented in the rumen; $AMCP^{DVE}$, truly absorbed microbial protein in the small intestine; ENDP, endogenous protein in the small intestine; dRUP, estimated intestinal digestibility of RUP; $ARUP^{DVE}$, truly absorbed rumen undegraded protein in the small intestine; DVE, truly digested protein in the small intestine; OEB, degraded protein balance; $AMCP^{NRC}$, truly absorbed microbial protein in the small intestine; $ARUP^{NRC}$, truly absorbed rumen undegraded protein in the small intestine; AECP, truly absorbed endogenous protein in the small intestine; MP, metabolizable protein; DPB^{NRC}, degraded protein balance.

4.3.4. Using protein spectral parameters as predictors of metabolizable protein characteristics (protein profiles, CNCPS subfractions, *in situ* rumen undegraded protein, estimated intestinal RUP degradability and predicted nutrient supply to dairy cattle)

The previous correlation study (Tables 4.4-4.5) showed that the simple correlation between protein structure spectral parameters and nutrition profiles in DDGS was weak. This led to the decision to examine multiple regression. The results obtained from multiple regression with variable selection are shown in Tables 4.6-4.8. The regression equations included amide profiles, α-helix, β sheet and their ratio. For protein profiles, the amide I to amide II ratio is a better predictor for crude protein (accounting for 79% of total variance), and neutral detergent insoluble protein (accounting for 72% of total variance). For CNCPS protein subfractions, amide I to amide II ratio can be used as a predictor of PB3 (with 61% of the variance being accounted for). To predict total digestible crude protein, amide I to amide II ratio accounts for 78% of the total variance. For RUP^{DVE} and RUP^{NRC} (g/kg DM), amide I to amide II ratio is a better predictor which accounts for 77% of the total variance. For *in vitro* estimation of intestinal RUP digestibility, amide I to amide II ratio is the only variable left in the model but only accounts for 23% of the total variance. In terms of protein supply, amide I to amide II ratio is the only significant predictor for $ARUP^{DVE}$, DVE, OEB, $ARUP^{NRC}$, AECP and MP values, while α-helix to β sheet intensity ratio can be solely used to predict $AMCP^{NRC}$ and DPB^{NRC} with the amide I to amide II ratio.

4.3.5. Use of DRIFT spectroscopy with multivariate molecular spectral analysis to distinguish differences in protein molecular structure among different DDGS and among original cereal grains

Univariate analysis for investigating the protein structural difference between cereal grains and different DDGS sample is not always capable of revealing all the differences. The accuracy of univariate analyses greatly depends on the functional group location and band patterns (Yu, 2005a). For example, both structural carbohydrate and non-structural carbohydrate show a peak at ca. 1180-950 cm^{-1} region (Wetzel et al., 1998). It is difficult to distinguish them using univariate analysis (Yu, 2005a). Multivariate analysis is an excellent tool for qualitatively

separating different groups of samples without knowing specific spectral assignments. Amide I and amide II region (ca. 1718-1488 cm^{-1}) was used to conduct the multivariate spectral analyses.

The cluster analysis of the spectral of the three original cereal grains (wheat, corn, and triticale) is shown in Figure 4.2. Paired comparisons between each pair of cereal grains were carried out. The mixed dendrogram of wheat and corn showed similarity of spectral data in amide I and amide II region, indicating wheat and corn were not completely different in protein spectroscopic features. Comparing wheat with triticale [Figure 4.2 (III)], wheat spectra were evenly mixed with triticale in the cluster dendrogram. These results clearly showed the similarity of wheat and triticale in protein spectral features. Given that triticale was genetically derived from wheat, this result was expected. In contrast, corn was well separated from triticale within linkage distance 1.5. This result showed that corn had a different spectroscopic feature in contrast with triticale in the amide I and amide II regions.

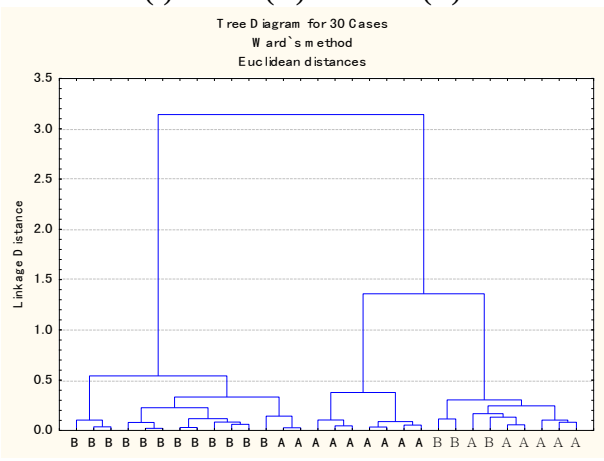
The paired comparisons among the three cereal grains (wheat, corn and triticale) and their DDGS (wheat DDGS, corn DDGS and triticale DDGS) using cluster analyses are presented in Figure 4.3. Wheat spectra were almost separated from wheat DDGS spectra within the linkage of 5 except for 4 spectra that were mixed with the wheat cluster [Figure 4.3 (I)]. For corn and corn DDGS, the spectra in the amide I and amide II regions were also clearly separated from each other. Similarly, triticale and triticale DDGS were different in protein spectral features in the amide I and amide II region. These results [Figure 4.3 (III)] were consistent with Yu et al. (2010). These results suggest that bioethanol processing changed the protein spectral profiles of the DDGS regardless of the substrate (wheat, corn and triticale). Doiron et al. (2009b) reported that clusters were observed between raw flaxseed and heated flaxseed under certain temperatures and heating times. These results indicate that bioethanol processing is the reason for the different protein spectral features for DDGS and original grains, and multivariate molecular analysis is able to detect the processing-induced changes in protein structure.

Among the three types of DDGS (wheat DDGS, corn DDGS and triticale DDGS), paired comparisons were carried out in order to determine differences in their protein structure (Figure 4.4). According to the dendrograms, wheat DDGS spectra were not completely distinguished from corn and triticale DDGS, because the spectra mixed with each other and no effective

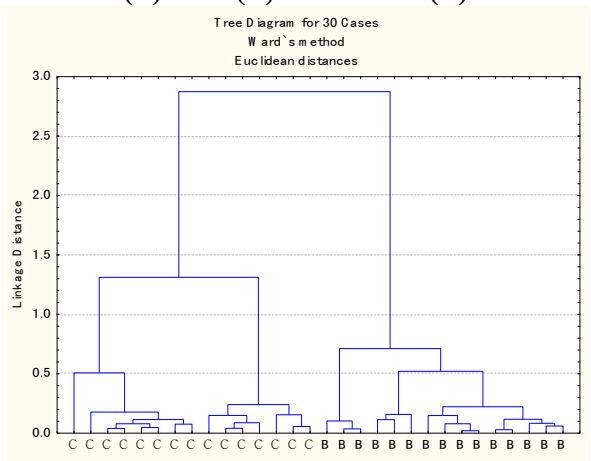
grouping was found. However, between corn and triticale DDGS, most of the spectra were separated and displayed in two groups. The separation between corn and triticale DDGS was similar to that between corn and triticale grains.

Cluster Analysis (CLA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat (A) and corn (B)



(II) Corn (B) and triticale (C)



(III) Wheat (A) and triticale (C)

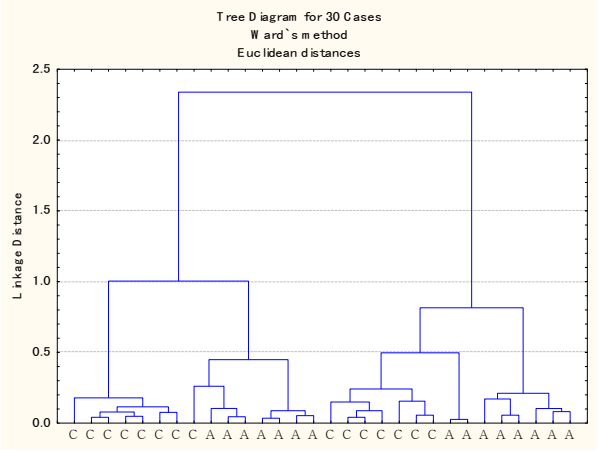
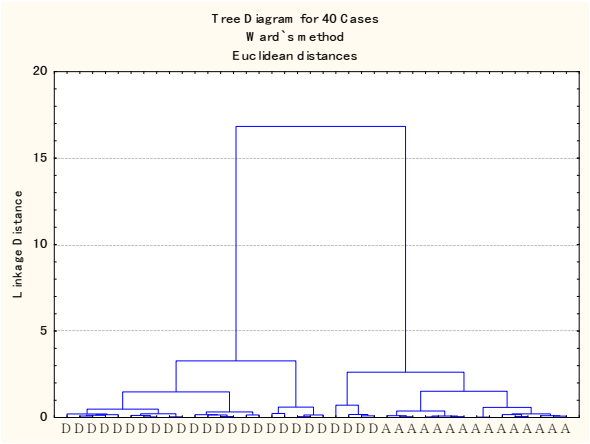


Figure 4.2 Multivariate molecular spectral analysis (CLA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) of grains: (I) Comparison of wheat and corn; (II) Comparison of corn and triticale; (III) Comparison of wheat and triticale

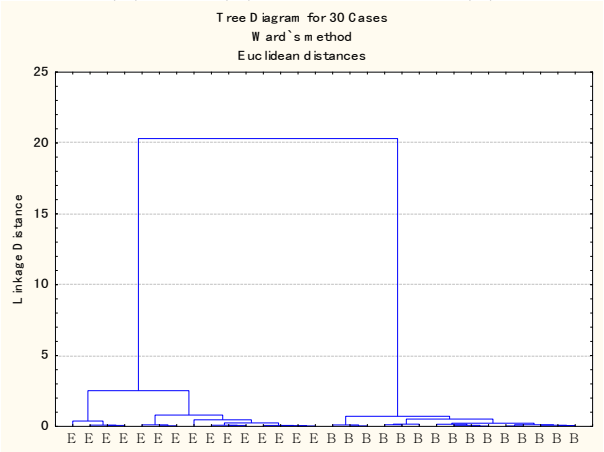
Cluster analysis (CLA): (1) Select spectral region: Amide I and II region: ca. 1718-1488 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm.

Cluster Analysis (CLA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat (A) and wheat DDGS (D)



(II) Corn (B) and corn DDGS (E)



(III) Triticale (C) and triticale DDGS (F)

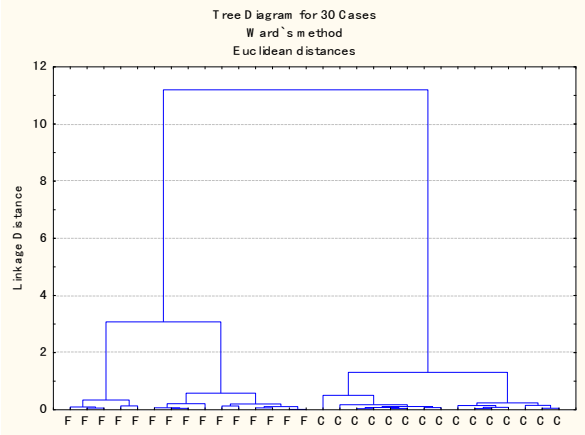
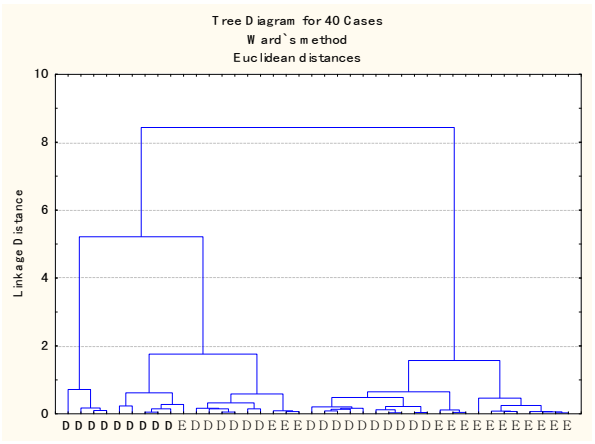


Figure 4.3 Multivariate molecular spectral analysis (CLA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) of original grains and their dried distillers grains with solubles (DDGS): (I) Comparison of wheat and wheat DDGS; (II) Comparison of corn and corn DDGS; (III) Comparison of triticale and triticale DDGS

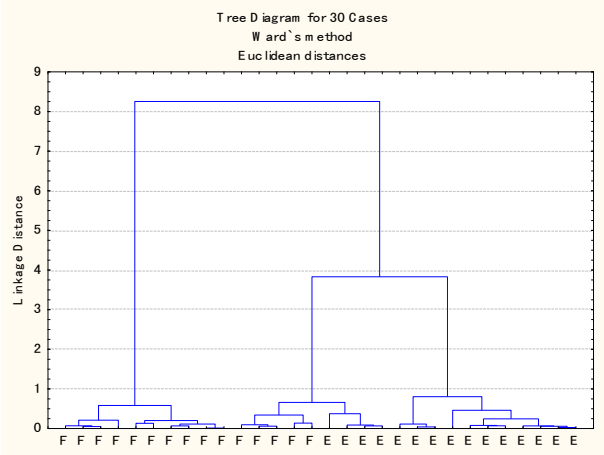
Cluster analysis (CLA): (1) Select spectral region: Amide I and II region: ca. 1718-1488 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm.

Cluster Analysis (CLA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat DDGS (D) and corn DDGS (E)



(II) Corn DDGS (E) and triticale DDGS (F)



(III) Wheat DDGS (D) and triticale DDGS (F)

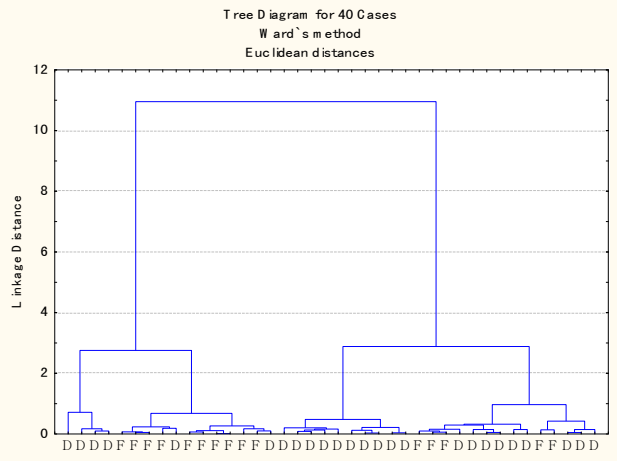


Figure 4.4 Multivariate molecular spectral analysis (CLA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) between different dried distillers grains with solubles (DDGS) : (I) Comparison of wheat DDGS and corn DDGS; (II) Comparison of corn DDGS and triticale DDGS; (III) Comparison of wheat DDGS and triticale DDGS

Cluster analysis (CLA): (1) Select spectral region: Amide I and II region: ca. 1718-1488 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm.

4.4. Conclusion

These results showed that bioethanol processing changed the protein molecular structure. The differences in protein molecular structure in cereal grains and their DDGS cannot be detected using traditional wet chemical analysis or other chemical based feed evaluation methods. With the application of Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) and Synchrotron Based Fourier Transform Infrared Microspectroscopy (SFTIRM), along with univariate and multivariate analyses, information on the quantitative and qualitative protein molecular structure can be obtained. The protein molecular structure was correlated to the chemical composition, rumen degradation kinetics, intestinal protein availability and protein supply to dairy cattle in this study. The prediction from protein molecular structure to protein digestive characteristics in dairy cattle is possible. DRIFT and SFTIRM together can give comprehensive protein molecular structure information from both ground and intact samples in a cereal grain. Although there might be differences between spectra obtained by the two techniques, the results are correlated to each other. This suggests the feasibility of using DRFIT and SFTRIM together in further cereal grain studies.

5. General Discussion and Conclusions

This study investigated a relatively new cereal grain namely triticale. Due to less competition from human consumption, triticale is potentially of greater value for bioethanol production than wheat. The results showed that the chemical composition of triticale was greatly concentrated by bioethanol processing except for starch. There was almost a 90% decrease in starch, about a 60% increase in crude protein and about a 77% increase in ether extract from triticale grain to triticale dried distillers grains with solubles (DDGS). The results also showed that triticale had a similar nutrient value (e.g. crude protein and starch) to wheat grain. This similarity was not changed by bioethanol processing, since the nutrient value of DDGS obtained from wheat and triticale were similar. In addition, the ethanol yield from wheat is comparable to that from triticale. CNCPS subfractions data showed triticale DDGS contained significantly higher PA (non-protein nitrogen) and PC (undegradable protein) fractions than triticale. This indicates that the protein fractions that are associated with specific degradation characteristics are changed by bioethanol processing. For energy values, there was no significant improvement from triticale to triticale DDGS, which suggests that triticale DDGS is an excellent alternative energy source for ruminants.

The overall results from this study suggest that there are significant differences between triticale grain and triticale DDGS. The nutritional value, CNCPS carbohydrate and protein subfractions, *in situ* rumen degradation kinetics, and predicted nutrient supply to dairy cattle of triticale DDGS were significantly different from triticale after bioethanol processing. These differences were not only related to changes in chemical composition but were also related to changes in protein molecular structure. Using molecular infrared spectroscopy techniques such as Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) and Synchrotron Based Fourier Transform Infrared Microspectroscopy (SFTIRM), along with univariate and multivariate spectra analyses, the structural changes in protein from cereal grains to their DDGS have been measured quantitatively and qualitatively. There were significant correlations between protein digestive characteristics and protein molecular structure. A regression model was tested and prediction equations were established using amide I and amide II peak areas, the ratio of amide I to amide II, α helix and β sheet peak height, and the ratio of α helix to β sheet to predict the protein degradation and nutrient supply characteristics for dairy cattle.

Previously published studies have reported that differences in protein digestibility are correlated to acid detergent insoluble crude protein (ADICP) which is an unavailable form of protein for ruminants. Therefore, knowing how much ADICP is in the total crude protein and its ruminal and postruminal availability for different DDGS is important. Few comparisons between the ADCIP content of triticale and triticale DDGS are available. The information contained in the current study may contribute to the modification of bioethanol processing procedures in the production of triticale DDGS. The rumen *in situ* data suggest that triticale has a higher rumen undegraded protein (RUP) content (ca. 40% improvement) than triticale DDGS. *In vitro* results showed that intestinal digestibilities of RUP for triticale and triticale DDGS were similar. However, the total digestible feed protein in triticale DDGS was higher than that in triticale. It can be concluded that triticale DDGS is superior to triticale not only in crude protein content but also in total digestible feed protein content.

The DVE/OEB and NRC-2001 models can predict potential protein supply to dairy cattle by combining the synthesized microbial protein, truly absorbed rumen undegraded protein and endogenous protein (gains or losses). The results showed that triticale DDGS was significantly higher than triticale in total truly absorbed protein in the small intestine. The degraded protein balance was positive in triticale DDGS but negative in the triticale grain. The results indicate there is potential N loss from rumen microbial protein synthesis in triticale DDGS. The negative protein degradation balance in triticale suggests that triticale did not provide sufficient rumen degraded protein for rumen microbial synthesis.

Chapter 4 reports the results of the protein molecular structure determination in cereal grains such as wheat, corn and triticale and their DDGS. The univariate analysis quantitatively measured peak intensity by integrating the peak area or height under specific protein sensitive bands. Grains were well separated from DDGS when comparing their amide profiles as well as their α helices, β sheets and the ratio between them. There were significant differences among several spectral parameters for the different cereal grains. In addition, significant differences were found in the amide I to amide II ratio and the α helix to β sheet ratio between the spectra obtained by DRIFT and SFTIRM. This is due to the different samples and regions where the spectra were obtained. A correlation between protein molecular structure and chemical profiles and CNCPS subfractions and nutrient supply predictions was confirmed. For example, the amide

I to amide II ratio was negatively correlated to rumen undegraded protein, the truly absorbed protein in the small intestine (DVE/OEB) and degraded protein balance (using both DVE/OEB and NRC-2001). Regression equations were successfully established based on the protein digestive characteristics and protein molecular structure parameters. For the DVE/OEB 1994 model, one of the best prediction equations was the truly absorbed protein in the small intestine (DVE) = 296.17 – 38.98 × the amide I to amide II ratio (R^2 = 0.72). For NRC-2001 system, one of the best prediction equations was the metabolizable protein (MP) = 300.96 – 43.32 × the amide I to amide II ratio (R^2 = 0.76).

These results suggest that the protein molecular structure parameters can be used as predictors to evaluate protein digestive characteristics. The findings of this study may benefit ethanol producers, feed companies, seed breeders and animal nutritionists in many ways. For ethanol producers, this study revealed that Canadian triticale has a high starch content, and this fundamental characteristic affects ethanol production. In addition, another important economic consideration for ethanol producers is the value of dried distillers grains with solubles (DDGS) as a byproduct. This study demonstrated that triticale DDGS has great potential to be a very good feed ingredient because it contains a high protein and high available fiber content. The high protein content in triticale DDGS provides sufficient nitrogen for ruminants to synthesize microbial protein which is a great contributor to milk and beef production. The highly available fiber content in triticale DDGS makes it a potential energy source for ruminants since ruminants have the ability to digest and convert available fiber to energy via microbial fermentation. This energy from fiber digestion directly contributes to the synthesis of microbial protein.

The potential for the DRIFT technique in detecting the nutritional value and the protein digestive characteristics of DDGS was confirmed in this study. In order to improve the nutritional value of DDGS, ethanol producers may utilize the DRIFT technique to measure the effect of different ethanol processing procedures (e.g. pH, temperatures, heating methods and durations) on the nutritional value of DDGS (e.g. metabolizable protein). Metabolizable protein is the sum of all available proteins that can be absorbed by ruminants and it is an important indicator of dairy cattle production performance. Therefore, knowing the amount of metabolizable protein is extremely important to fulfill an animal's requirements and formulate accurate rations. Traditionally, to estimate this metabolizable protein value, a series of time-

consuming and expensive methods such as chemical analyses, animal experiments and sophisticated modeling have been used. However, DRIFT can greatly simplify theses analytical procedures by detecting the amide I to amide II ratio. According to this study, a lower amide I to amide II ratio indicates a higher metabolizable protein content. Therefore, an ethanol producer may modify the ethanol processing procedures simply based on the amide I and amide II ratio to optimize the quality of DDGS. Another indicator, the α helix and β sheet ratio is less useful compared with amide I to amide II ratio because of its correlation coefficient is relatively lower.

For feed companies, the near infrared spectroscopy technique has been extensively used to detect the chemical composition on site because it is rapid, cheap and efficient. Similarly, the DRIFT spectroscopy using mid infrared may also be applied by feed companies to rapidly detect protein degradability. Since a higher amide I to amide II ratio indicates a higher rumen undegradable protein content, time consuming chemical analyses and expensive animal trials may be not required. Feed companies may save a large amount of time and money by applying the DRIFT technique. The application may greatly improve the efficiency of quality control and ration formulation for feed companies and eventually benefit dairy producers. However, to increase the accuracy of DRIFT techniques, a greater number of feedstuffs should be investigated in future studies in order to establish a database and develop more accurate prediction equations.

Compared with the DRIFT technique, the SFTIRM technique is also an excellent tool to discover changes in protein structure at both the molecular and cellular levels because of its superior accuracy in the spectra collection. Technically, the SFTIRM technique can reveal the molecular structure on specific spots of a cross section of a seed. It can also give a colored mapping which shows the visualized distribution of different chemical compounds. This is very useful to identify the locations of a chemical compound of interest in a seed. For example, using these spectrum and mapping data, seed breeders can identify seeds with higher metabolizable protein (based on their lower amide I to amide II ratio) and further locate the accurate positions of these metabolizable protein rich areas. With the help of visualized mapping, seed breeders can develop new plant varieties by investigating which variety has a lower amide I to amide II ratio using SFTIRM. It can be imagined that once a new seed variety with a high metabolizable protein portion is developed, it may be possible to separate the most nutritious parts of the seeds

by seed processing companies to generate a new dairy feed with concentrated metablizable protein. In future studies, this techniques can also be expanded to food research. It can be used to detect the differences between different varieties of seeds targeting specific nutritional values (e.g. carbohydrate, fiber and lipids) which are important factors in human nutrition.

For animal nutrition studies, the advantages and disadvantages of DRIFT and SRFIRM techniques should be considered when conducting research. According to this study, the results obtained from the DRIFT and SRFITRM techniques are different but are highly correlated in determining protein structures. Considering that the SFTIRM technique is more expensive and only available for use with intact seed cross section samples, the DRIFT technique has greater potential for a wider scale of applications in feed science because it is capable of analyzing different forms of feed. However, when more accuracy is needed in the nutritional study of seeds, the SFTIRM technique should be considered.

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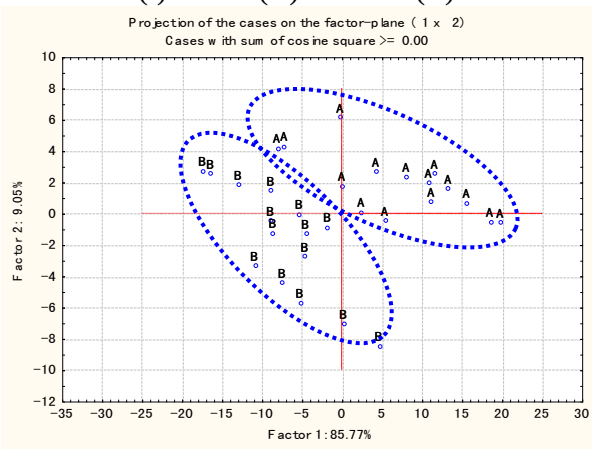
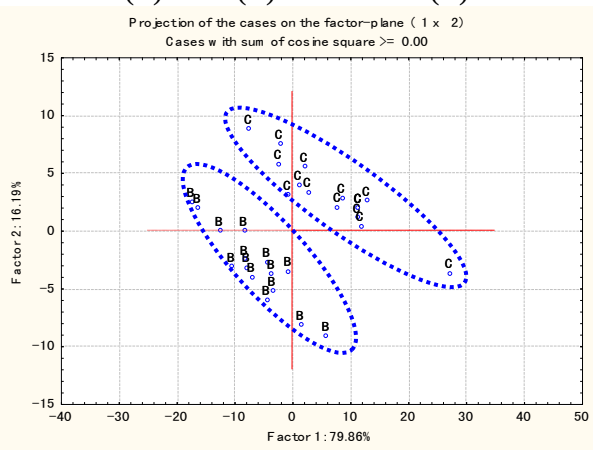
7. Appendix

Principal Component Analysis (PCA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat (A) and corn (B)



(II) Corn (B) and triticale (C)



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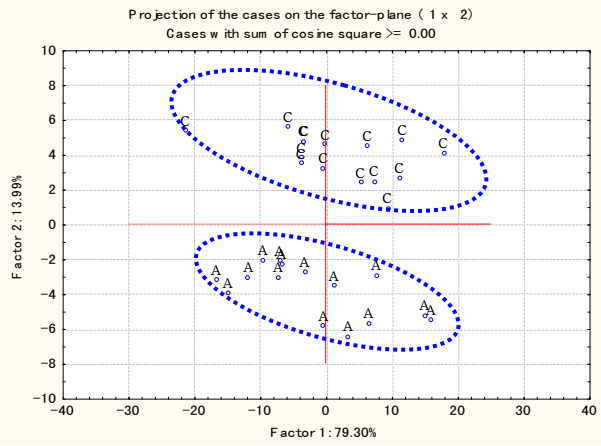
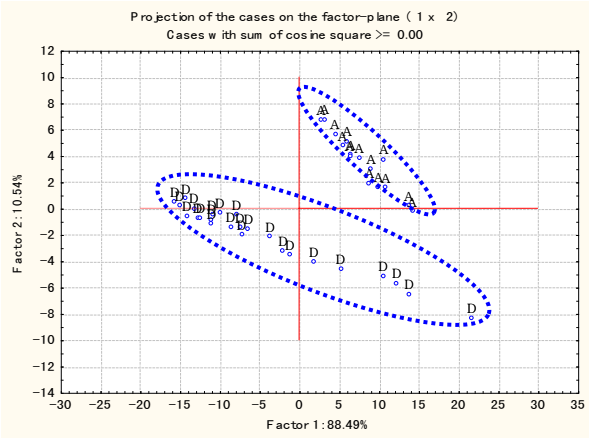


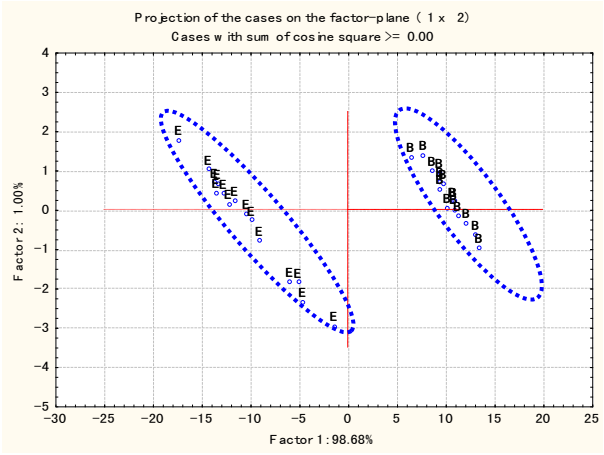
Figure 7.1 Multivariate molecular spectral analysis (PCA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) of grains: (I) Comparison of wheat and corn; (II) Comparison of corn and triticale; (III) Comparison of wheat and triticale
Principal component analysis (PCA): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Principal Component Analysis (PCA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat (A) and wheat DDGS (D)



(II) Corn (B) and corn DDGS (E)



(III) Triticale (C) and triticale DDGS (F)

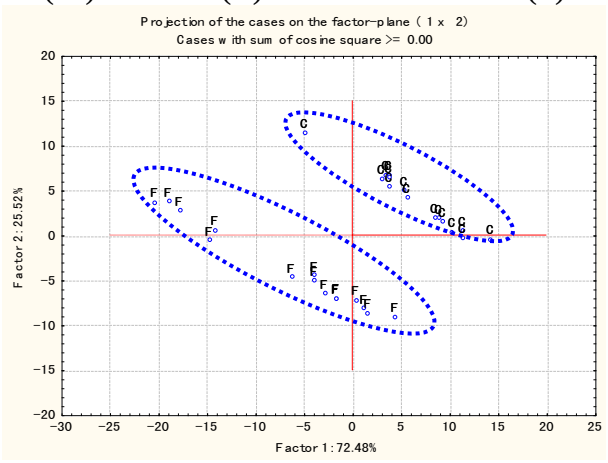
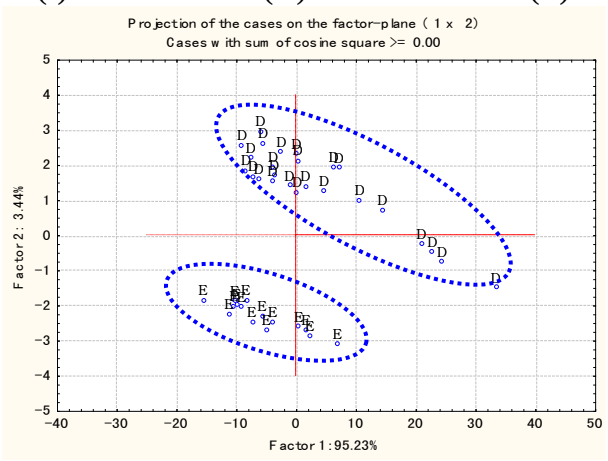


Figure 7.2 Multivariate molecular spectral analysis (PCA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) of original grains and their dried distillers grains with solubles (DDGS): (I) Comparison of wheat and wheat DDGS; (II) Comparison of corn and corn DDGS; (III) Comparison of triticale and triticale DDGS

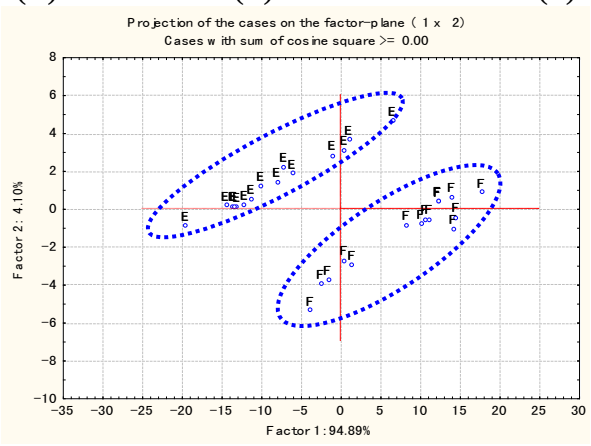
Principal component analysis (PCA): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Principal Component Analysis (PCA): Amide I and II Regions ca. 1718-1488 cm^{-1}

(I) Wheat DDGS (D) and corn DDGS (E)



(II) Corn DDGS (E) and triticale DDGS (F)



(III) Wheat DDGS (D) and triticale DDGS (F)

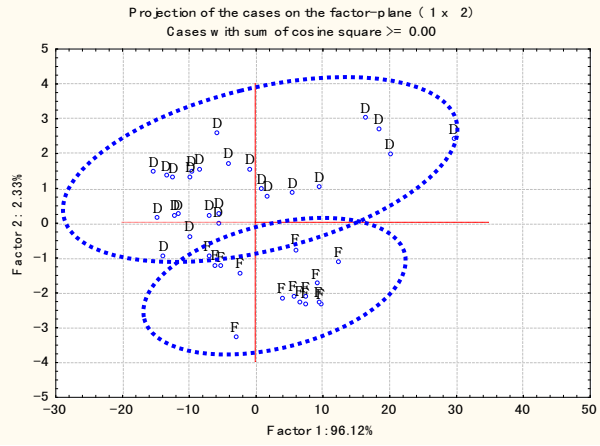


Figure 7.3 Multivariate molecular spectral analysis (PCA) of the amide I and II regions (ca. 1718-1488 cm^{-1}) between different dried distillers grains with solubles (DDGS) : (I) Comparison of wheat DDGS and corn DDGS; (II) Comparison of corn DDGS and triticale DDGS; (III) Comparison of wheat DDGS and triticale DDGS

Principal component analysis (PCA): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

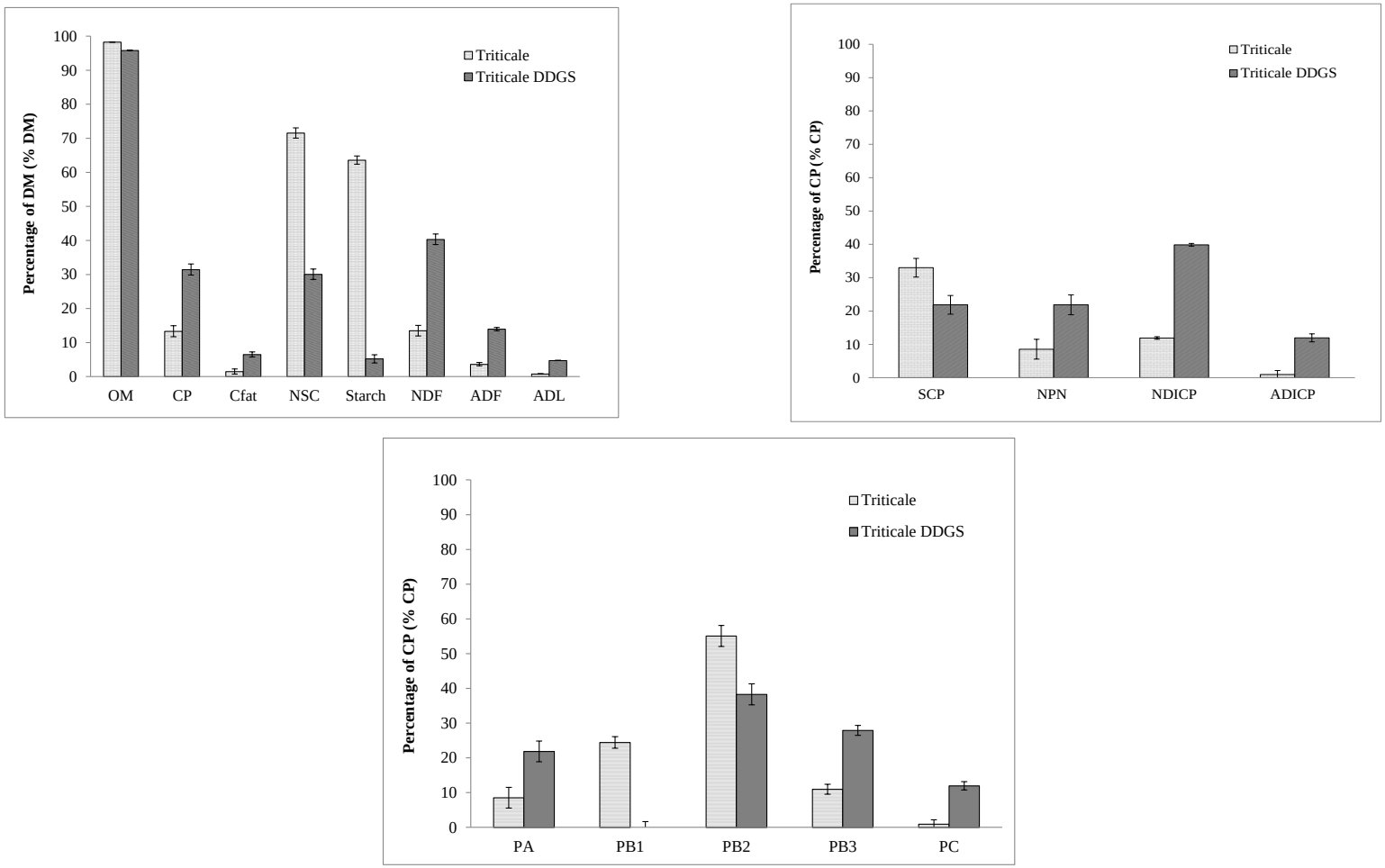


Figure 7.4 Chemical profiles and protein fractions of triticale grain and dried distillers grains with solubles (DDGS) determinted with the Cornell Net Carbohydrate and Protein System

Abbreviations: OM, organic matter; CP, crude protein; EE, ether extract; NSC, non-starch carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; SCP, soluble crude protein; NPN, non-protein nitrogen; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; PA, non-protein nitrogen; PB1, rapidly degradable protein fraction; PB2, intermediately degradable protein fraction; PB3, slowly degradable protein fraction; PC, unavailable protein fraction.

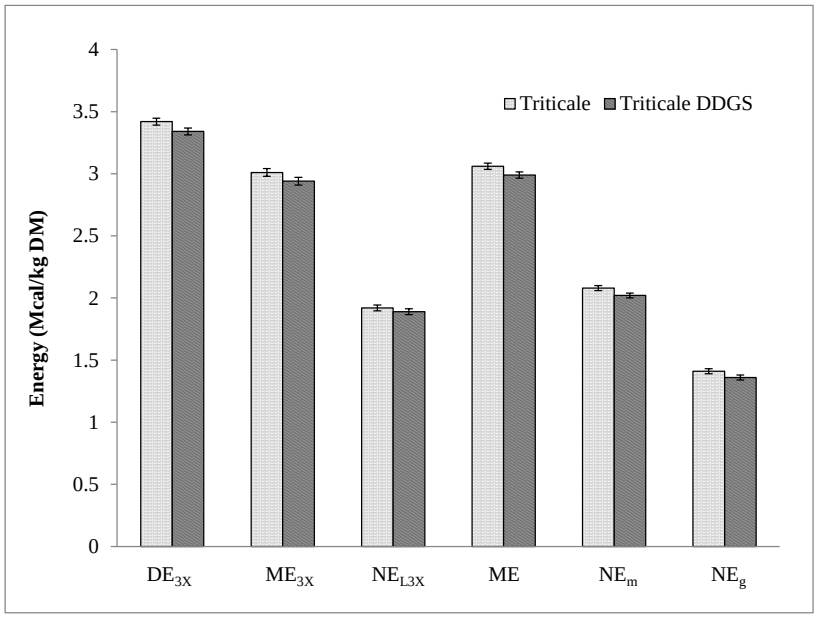


Figure 7.5 Energy content of triticale grain and dried distillers grains with solubles (DDGS) Abbreviations: DE_{3X}, digestible energy at three times maintenance; ME_{3X}, metabolizable energy at three times maintenance; NE_{L3X}, net energy for lactation at three times maintenance; ME, metabolizable energy; NE_m, net energy for maintenance in growing animals; NE_g, net energy for growth.

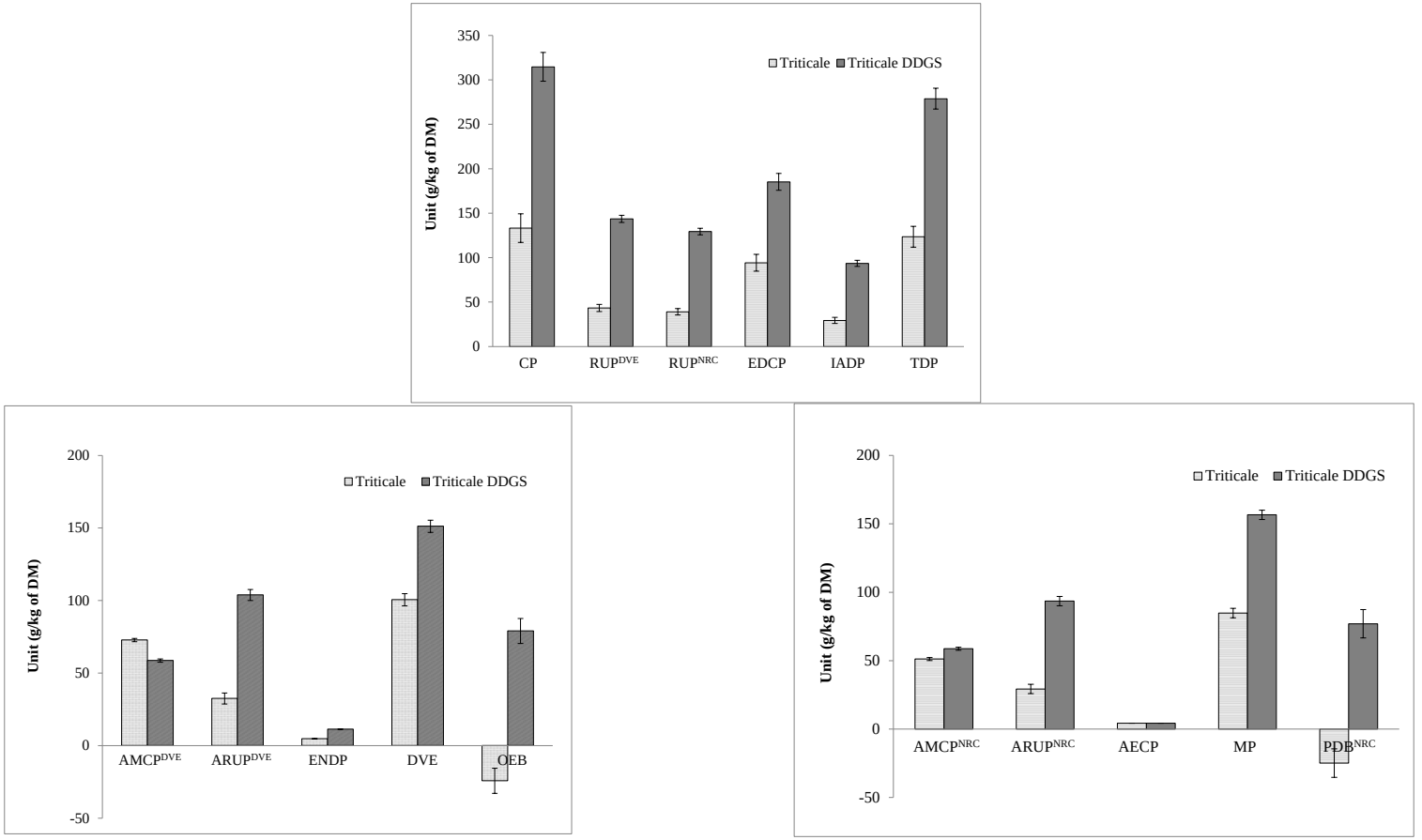


Figure 7.6 Intestinal protein digestibility and nutrient supply of triticale grain and dried distillers grains with solubles (DDGS) predicted using the DVE/OEB system and the NRC-2001 model

Abbreviations: CP, crude protein; RUP^{DVE}, rumen undegraded feed protein (DVE/OEB model); RUP^{NRC}, rumen undegraded feed protein (NRC 2001 model); EDCP, effective degradation of feed CP; IADP, estimated intestinally absorbable feed protein; TDP, total digestible feed protein; $AMCP^{DVE\ or\ NRC}$, truly absorbed microbial protein in the small intestine (DVE/OEB or NRC 2001 model); $ARUP^{DVE\ or\ NRC}$, truly absorbed rumen undegraded protein in the small intestine (DVE/OEB or NRC 2001 model); ENDP, endogenous protein in the small intestine (DVE/OEB model); DVE, truly digested protein in the small intestine (DVE/OEB model); OEB, degraded protein balance (DVE/OEB model); AECP, truly absorbed endogenous protein in the small intestine (NRC 2001 model); MP, metabolizable protein (NRC 2001 model); DPB^{NRC}, degraded protein balance (NRC 2001 model).

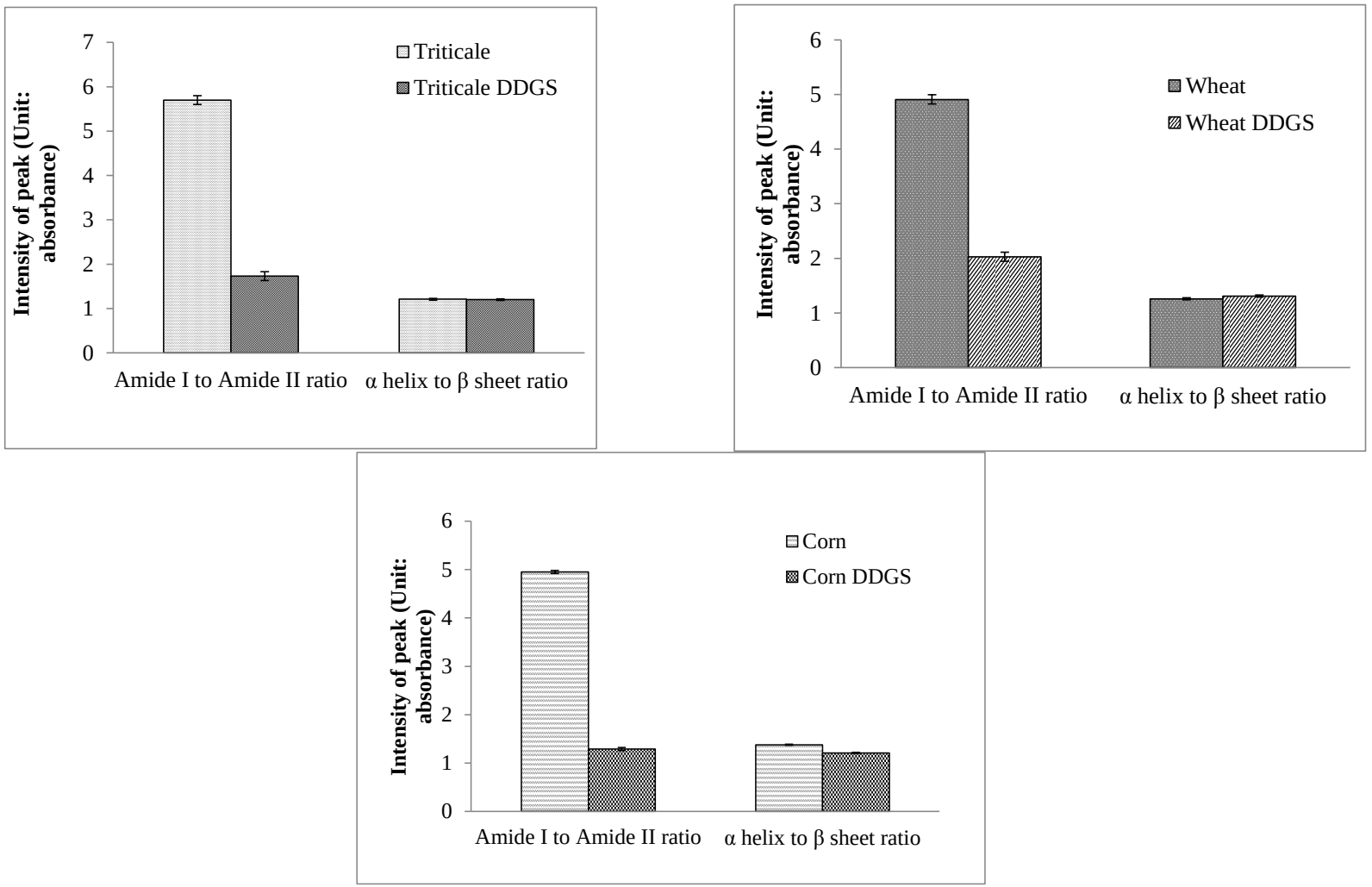
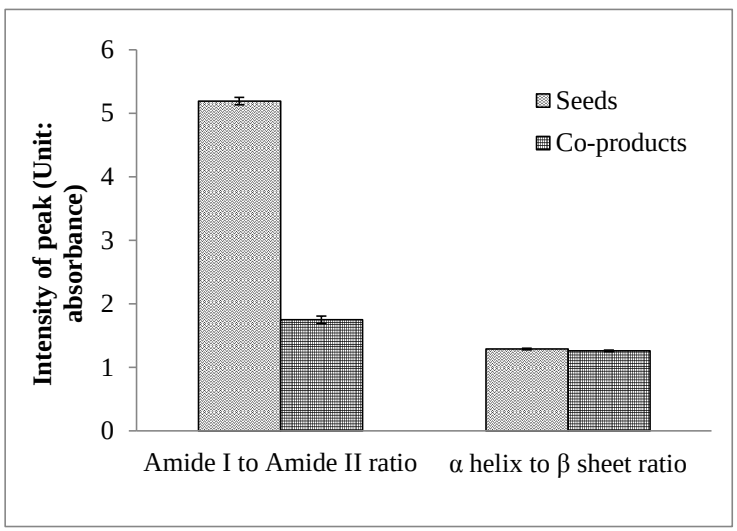
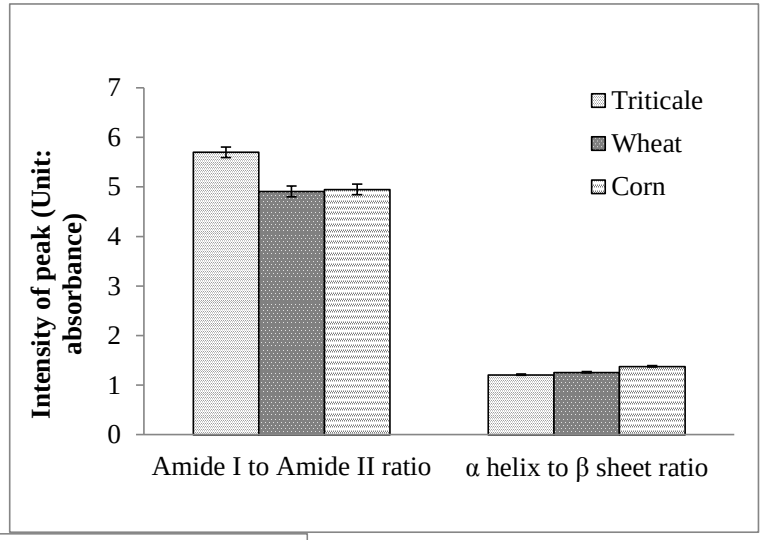


Figure 7.7 Amide I to amide II ratio and α helix to β sheet ratio of different cereal grains and their dried distillers grains with solubles (DDGS) collected using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy





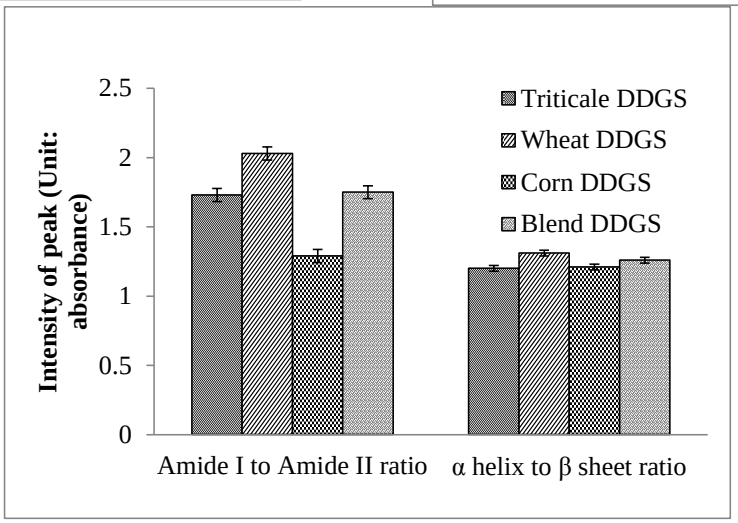


Figure 7.7 (*Continued*) Amide I to amide II ratio and α helix to β sheet ratio of different cereal grains and their dried distillers grains with solubles (DDGS) collected using Diffuse Reflectance Infrared Fourier Transform Spectroscopy and Synchrotron Based Fourier Transform Infrared Microspectroscopy